TOXICOLOGY OF PHENOXY HERBICIDES AND HAZARD ASSESSMENT OF THEIR USE IN REFORESTATION

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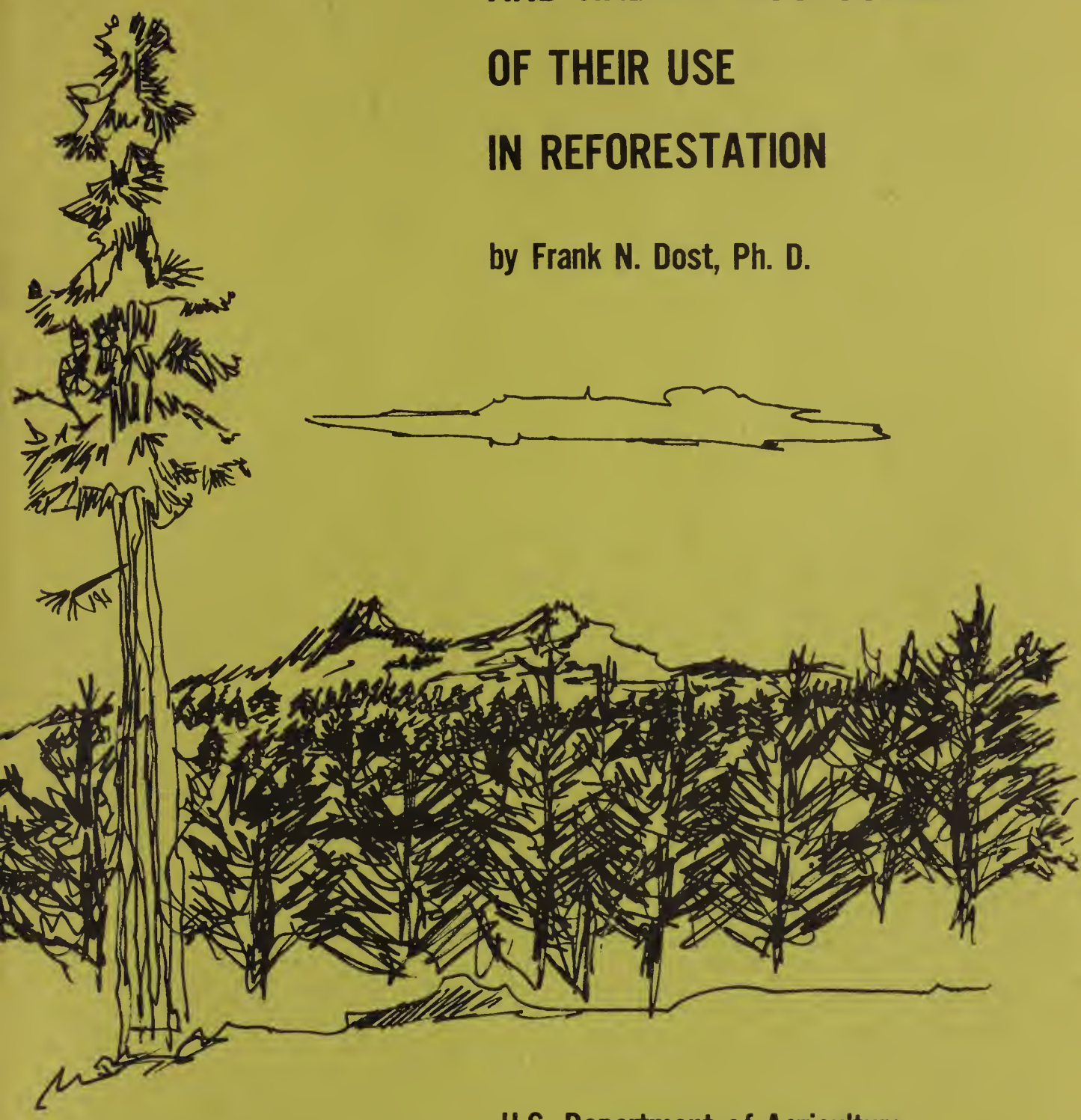

U.S. Department of Agriculture
Forest Service, California–Pacific Region

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By Frank N. Dost, Ph. D.

(Toxicologist, Associate Professor of Veterinary Medicine,
Environmental Health Science Center,
Oregon State University)

U. S. Department of Agriculture
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FOREWORD

This report was prepared by Dr. Frank N. Dost (Toxicologist, Assoc. Prof. Vet.Medicine, Environmental Health Science Center, Oregon State University) under a contract with the U.S. Forest Service. The report was designed to provide a comprehensive evaluation of the hazard to human health of phenoxy herbicides, specifically to include 2,4-D, 2,4,5-T, and Silvex as used in forest, range, and brushland management on the National Forests in California.

Dr. Dost reviewed our present environmental statements on forest reestablishment, rangeland enhancement, and brushland management and analyzed risks and provided suggestions on how to best minimize hazards to human health. He reviewed all available published and unpublished reports on research and studies of herbicide effects. His work was reviewed by 16 scientists in related fields, and their comments were considered in his final report

This report is part of our current effort to do the best possible job in *updating* and, where appropriate, *revising* the Forest Service environmental statements for forest reestablishment, rangeland enhancement, and brushland management.

Douglas R. Leisz

DOUGLAS R. LEISZ
Regional Forester

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Dr. Hugh Black, Associate Professor
Forest Wildlife Ecology
Dept. of Forest Management
Oregon State University
Corvallis, Oregon 97331

Dr. R. K. Boutwell
Professor of Oncology
McArdle Laboratory for Cancer Research
University of Wisconsin
Madison, Wisconsin 53706

Dr. Don Crosby
Dept. of Enviromental Toxicology
University of California
Davis, California 95616

Ephraim Kahn, M. D., Chief
Dept. of Public Health
Epidemiological Studies Lab
2151 Berkeley Way
Berkeley, California 94794

William Griffith
California Dept. of Fish & Game
987 Jedsmith Drive
Sacramento, Ca. 95819

Dr. Keith Maddy
California Department of Food & Agriculture
1220 N. Street
Sacramento, California 95814

Dr. Wilbur McNulty
Oregon Regional Primate Center
505 N. W. 185 Avenue
Beaverton, Oregon 97005

Thomas Milby, M.D.
Stanford Research Institute
2150 Shattuck Avenue
Berkeley, California 94706

Donald Morgan, M.D.
Agricultural Medical Institute
Oakdale, Iowa 52319

Dr. Logan Norris
U.S. Forest Service, PNW
3200 Jefferson Way
Corvallis, Oregon 97331

Dr. Mike Newton
Dept. of Forest Mgt.
Oregon State University
Corvallis, Oregon 97331

Dr. Steve Radosevich
Dept. of Botany
University of California
Davis, California 95616

Dr. Rojer Sandquist
U.S. Forest Service
Region 6

Fay Shon
U.S. Forest Service, PNW
3200 Jefferson Way
Corvallis, Oregon 97331

Dr. Brian Sturgess
U.S. Forest Service
Region 5

Dr. James Witt, PH.D.
3223 N.W. Glywood Circle
Corvallis, Oregon 97330

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Introduction

The phenoxy herbicides 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), 2,4-dichlorophenoxyacetic acid (2,4-D), and 2,4,5-trichlorophenoxypropionic acid (silvex) are important agents for reforestation site preparation and for release of conifer plantings. To be used, the compounds must be inserted into a segment of the environment that may be occupied by humans or may have a potential for migration to points of human contact or consumption. This document is a review of the pertinent literature describing biological effects and environmental behavior of the three herbicides, and of 2,3,7,8-tetrachlorodibenzo-p-dioxin, an extremely toxic contaminant found in small quantities in 2,4,5-T and silvex.

For various reasons, the greatest public attention has been focused on 2,4,5-T and its unique contaminant, TCDD. Since 1969 public and scientific attention to TCDD has overshadowed the specific concerns about 2,4-D, which has no TCDD, and about 2,4,5-T and silvex, which are contaminated.

The unusual nature of the TCDD contaminant has made a special case of the herbicide 2,4,5-T, and has made it the subject of an unusually vehement public, political, and scientific controversy. An enormous scientific effort has evolved in an attempt to learn about the unique character of the compound, and to provide a rational basis for regulatory decisions. Hopefully, this effort to bring the existing literature into focus will aid in the decision-making process.

As matters presently stand, there is a division in the collective public and scientific minds on the safety and benefits of use of the phenoxy herbicides. As in any controversy, both views include unreasonable people, but the bulk of the argument is among sincere citizens and competent scientists on either side. It is my hope that the reviews and assessments in this document are dispassionate and without bias; otherwise it will be of limited utility.

In preparing this review, emphasis has been placed on information about the toxic properties of the respective agents. An assessment of hazard necessarily considers along with biological effects, factors governing the probability of exposure, and the probability that the chemical once contacted will enter the organism.

With the exception of the section on TCDD, I have chosen to touch only lightly upon the environmental behavior of the compounds. This information about phenoxy herbicides is well established and is much less complex than the behavior of TCDD. A more thorough treatment of the environmental behavior of TCDD is necessary here because: 1) this contaminant is clearly the most important of the four compounds under study; 2) the high toxicity demands thorough understanding of the compound as it exists outside the target of toxic effect; 3) the environmental chemistry of TCDD is incompletely studied and subject to some controversy, whereas that of the herbicides themselves is well established.

The organization of this review and assessment includes sections reviewing the scientific literature on TCDD, 2,4,5-T, 2,4-D, and 2,4,5-TP (silvex), an assessment of the hazard potential of their distribution in the environment, recommendations on some aspects of methods of use of

the herbicides and recommendations of needed research. A shortened version of the review and assessment is also attached. Organization within the review differs slightly among the chemical sections because of differences in research emphasis or the character of the agent itself. In certain instances, for example in considering the enzyme induction capacity of TCDD, an area may be dealt with that is not entirely germane to the issue of hazard but of considerable importance in understanding the biology of the compound.

A number of useful reviews of interaction of phenoxy herbicides or TCDD with animal systems have been or are being published. The most recent one by Leng (1977), discussing metabolic fate of phenoxy acids in domestic animals, Mercier (1976) in response to the Seveso accident which spread large amounts of TCDD through several Italian communities, and McQueen et al. (1977) refuting alleged association between herbicide use and fetal abnormalities in humans. McKenzie et al. (1975), Norris et al. (1972), Buhler (1977), and the EPA position paper arising from the Dioxin Working Group (presently in draft) are also all of considerable value.

TOXICOLOGY OF TCDD

History of Human Exposure

Probably the first clinical description of human TCDD exposure arose from the study by Kimmig and Schulz (1957) of workers in chlorophenol factories in Germany. The principal symptom was a persistent skin lesion (chloracne; so-called because it is characteristic of a number of related compounds and was at one time thought to be caused by free chlorine.

The active agent was established as TCDD, and animal studies showed that the same lesions appeared on rabbit ears after application of as

little as 0.002% TCDD (Kimmig and Schulz, 1957). High topical doses and oral doses of 50 µg/kg or greater caused liver necrosis. A similar industrial intoxication in France was reported by Dugois and Colomb (1957). Pathology of the skin lesions is well described by Kimbrough (1974).

A study of 29 phenoxy herbicide workers by Bleiberg et al. (1964) disclosed chloracne and a high frequency of uroporphyrinuria, and tissue deposition of porphyrins related with hyperpigmentation and skin fragility. This assemblage of symptoms is commonly called porphyria cutanea tarda. The excessive production and excretion of porphyrins apparently results from a hyperactivity of the enzyme δ-amino levulinic acid (ALA) synthetase, the first and rate limiting step in porphyrin formation. Porphyrins are eventual components of heme proteins, such as hemoglobin and cytochromes. Effects on ALA synthetase and other enzyme systems will be discussed in another section. Poland et al. (1971) examined the same factories later, after somewhat more satisfactory industrial hygiene measures were established. Among the 73 male workers observed, no clinical porphyria was apparent even though some degree of chloracne still persisted in most of the subjects. There was also apparently a significant psychological effect as measured by the Minnesota Multiphasic Personality Inventory.

The absence of porphyria and persistence of chloracne in the later study has led Poland et al. (1971) to conclude that while both may be caused by TCDD, the mechanisms are probably dissimilar.

Several severe accidental exposures to TCDD have occurred. In several cases, runaway chlorophenol plant reactions caused widespread exposures within the factory or in the surrounding community. An accident in Germany in 1953 exposed a large number of workers, said to have suffered liver, kidney, splenic, eye, cardiovascular, and nervous symptoms. A

worker who apparently was in extensive contact with a contaminated segment of the plant five years after the accident became ill and later died. An explosion in a Dutch plant in 1963 released 30-200 g of TCDD into the main gallery of the factory exposing 50 people. In the subsequent two years, four died, but no clear association with TCDD intoxication could be shown. (Hay, 1976). An explosion in a British plant in 1968 resulted in 79 cases of chloracne (May, 1973). In Czechoslovakia a pentachlorophenate production stream overheated under pressure, producing large amounts of TCDD (Jirazek et al., 1974). (It should be noted in this and some earlier German work a different numbering convention was used. 2,3,6,7-TCDD and 2,3,7,8-TCDD are the same compound.) Of 80 exposed workers, 76 developed chloracne, porphyria cutanea tarda, disorders of plasma lipids, hepatic damage, neural lesions, and neurasthenia.

An explosive reaction in a plant at Seveso, Italy, in 1976 scattered TCDD, apparently in kilogram amounts, over a wide area that included several communities. Details are still not widely disseminated but great numbers of animals were killed and many people were made ill. A review of the incident was prepared by Mercier (1976) a few months after the incident but the slow onset and persistent effects did not permit a detailed assessment of the damage. Since that time the Seveso disaster has been given unprecented publicity and as might be expected this tragedy has been sensationalized in press coverage, in part because there may have been an effort to minimize or cover up the extent of the problem. There were apparently defects in the administration of the plant that may have resulted in the accident itself, and management of public warning and information early after the accident was questionable. Further contributing to the extensive public attention and sensationalizing of

the incident is a virtual absence of objective reporting in the scientific literature. As far as I am aware, there has been no scientific evaluation of the aftermath in publication since the review by Mercier (1976), shortly after the accident.

In the U.S., TCDD extracted from hexachlorophene production was stored in oil, then mistaken for waste lubricating oil and sprayed on several horse arenas and farms in Missouri, for dust control. The incidents occurred in spring and summer of 1971. On one horse breeding farm, 62 of 85 horses exercized in the sprayed area became ill and 48 died, the last in January, 1974, two and a half years later. Hundreds of birds and rodents died, along with a number of dogs and cats. Several children were exposed through play in the arena soil. One developed a severe hemorrhagic cystitis and others showed clear evidence of chloracne. All recovered. The TCDD concentration in the soil was later established at more than 30 ppm, a truly massive amount. (This concentration amounts to 120 lb per acre per foot of depth, assuming 4×10^6 lb soil/acre-foot.) The details of the latter event are described in detail by Carter et al. (1975) and Kimbrough et al. (1977).

All of these exposures were to a mixture of TCDD with other agents. Possibly the only published account of exposure to pure TCDD is the description by Oliver (1975) of laboratory contact by three individuals associated with synthesis experiments. The individuals all had utilized routine protective procedures but sufficient contact developed nonetheless. The exposures were not sufficient to cause porphyrinuria or liver damage but serum cholesterol was raised. In two cases personality changes and neural disorders developed two years after exposure. The amount of TCDD contacted is not known.

General Toxicity in Laboratory Animals

This section is a review of data on effects of TCDD other than reproduction and teratology, carcinogenesis, and its enzyme inducing properties upon mammals. These will all be considered independently, as will studies of tissue distribution and metabolism.

Schwetz et al. (1973) have determined lethalities for several species. The guinea pig is exceedingly sensitive, with an LD_{50} of only 0.6 µg/kg; rats are less sensitive; LD_{50} for males is about 20 µg/kg and for females the LD_{50} is in excess of 40 µg/kg. Rabbits are still less sensitive at more than 100 µg/kg. Dogs were found to require even higher effective doses. In these studies, responses of mice were highly erratic and were not included. McConnell et al. (1977a) have determined a single 30 day LD_{50} for mice of 284 µg/kg, and 2 µg/kg for guinea pigs.

An important characteristic of TCDD is a very long delay before lethality, often 2-3 weeks and occasionally 7-8 weeks.

The pathology associated with TCDD toxicity may include thymic atrophy, liver necrosis, and degeneration in the kidney and thyroid. The guinea pig is unique in that it suffers limited hepatic damage, even at doses that cause substantial thymus depletion (Gupta et al., 1973). The degree of thymic atrophy is generally dose dependent; the studies of Gupta et al. (1973) did not establish a zero effect dose, but the lowest dose in the rat of 1 µg/kg/day for 31 days caused moderate thymus and liver damage, and kidney and thyroid degeneration. Guinea pigs treated with 0.2 µg/kg weekly for eight weeks had limited thymus damage and no other pathology. Studies of guinea pigs at lower doses suggest a no observed effect level at 0.008 µg/kg/weekly for eight weeks, a total of 0.064 µg/kg, although there was a slight decrease in lymphocytes at that dose (Vos et al.,

1973). Animals given tetanus toxoid at the fourth week to measure humoral immunity were found to have somewhat lower thymus weights than controls, although the difference was not significant.

According to the data of Kociba et al. (1976) the no observed effect level in rats given TCDD in the diet 5 days a week for 13 weeks is 0.001 µg/kg/ day, totaling 0.065 µg/kg, almost identical to the value found in guinea pigs. Again, thymus weight appeared to be the most sensitive parameter. The Kociba study dealt with a very broad range of clinical, chemical, and hematological measurements. At a dose of 1.0 µg/kg/day, 2 of 10 rats died and weight gain decreased sharply, erythrocyte numbers and volume decreased, reticulocytes increased in males. In females thrombocytes decreased and total leukocytes increased. Differential counts were not altered. Urinary porphyrins, creatine and δ amino levulinic acid (ALA) all increased. Serum bilirubin, BUN and alkaline phosphatase also were elevated. At an intake of 0.1 µg/kg/day red cell numbers and volume were still slightly decreased in males and porphyrin, ALA, bilirubin, and alkaline phosphatase was elevated in females. No changes in blood parameters occurred below these levels.

In a similar study Zinkl et al. (1973) found no changes in SGPT, bilirubin, or erythrocytes after 31 days of treatment at 1.0 µg/kg/day and no change in SGOT, cholesterol, or serum protein at 0.1 µg/kg/day. There was a modest depression in blood glucose at the lower dose rate.

Mice treated with 1.0 µg/kg/week for six weeks were found to have depleted thymus weight (Vos et al., 1974) but no changes were evident at the lowest dose, 0.2 µg/week. Hematological changes occurred only at a dose of 25 µg/kg/week, for six weeks. Some changes in serum proteins did occur at an intake of 0.2 µg/kg/weekly. Unfortunately, there is no data suggesting the magnitude of the no-effect single dose in any species.

Studies in primates have been limited. McNulty administered TCDD at a rate of 20 parts per billion in the diet to a single monkey and caused its death in a few days. A 10-fold lower concentration permitted survival for about two months. Total intake was estimated as about 6 µg/kg (McNulty, 1977). A study of eight female Rhesus monkeys fed 500 parts TCDD per trillion has recently been completed in the laboratory of J.R. Allen. The animals showed dermatitis and menstrual irregularities within six months after initiating treatment. One monkey died prior to the seventh month and by nine months of exposure, all survivors experienced a severe anemia and leukopenia. Food intake was normal but all of the animals lost weight. After 11 months, five of the eight monkeys died, all with severe anemia and destruction of blood forming tissues. The total dose was estimated at about three µg/kg (Allen et al., 1977a, 1977b) or about .3 µg/month.

A number of questions arise from these data. The monkey studies are limited, but they do suggest the possibility that the lethal dose is similar regardless of the time over which it is administered. The implications are quite interesting. If it is true that TCDD dosage is time independent in the monkey, then the effect is truly cumulative. No chemical is known to have such properties, and if TCDD behaves in this fashion, any estimate of acceptable dose must assume additive effects over long periods. TCDD does not remain in the body of monkeys (Van Miller et al., 1976) or other species (Van Miller et al., 1976; Fries and Marrow, 1975; Rose et al., 1976). A cumulative effect, if it exists, must then entail irreversible effects remaining after the molecule leaves active sites. TCDD is apparently not metabolized to a significant extent (Rose et al., 1976; Vinopal and Casida, 1973; Van Miller et al., 1976) so it is conceivable as well that a single molecule could interact several times before

leaving the body. A possible mechanism may be intercalation of the planar TCDD molecule into DNA (Hussain, 1973), production of misinformation and departure of the agent to interact at another DNA site.

Data in other species, especially the guinea pig do not support the idea of extensive cumulative effect. A dose rate of 0.2 µg/kg/weekly for eight weeks provided a total dose almost three times the LD_{50}, but no deaths occurred and organ changes, while measurable, were not severe (Vos et al., 1973). Rats also accepted about three times the LD_{50} of 20 µg/kg over 13 weeks, without lethality. Some mice, on the other hand, died at a total dose less than the acute LD_{50}, distributed over a four week period (Vos et al., 1974).

There are a few other biological responses to TCDD that appear after high doses of from 10-25 µg/kg. One of the more interesting is a depressed capacity for biliary excretion. TCDD sharply decreases removal of PCBs from the liver via the bile (Yang et al., 1977) and decreases plasma clearance of ouabain into bile (Hamada and Peterson, 1977). In the latter work, steroidal inducers of microsomal enzymes reversed the TCDD effect. Whether this effect has potential significance in the response to secondary toxicity can only be speculated upon. In any case the doses required for effect are quite high and probably not attainable under realistic environmental conditions.

Because TCDD causes increased microsomal foreign compound metabolizing activity in the kidney cortex (Fowler et al., 1975, 1977), Pegg et al. (1976) evaluated proximal tubular function in intoxicated rats. They found little change at any but large doses (25 and 50 µg/kg).

There have been few observations of metabolic changes caused by TCDD. Incorporation of ^{3}H-sodium acetate into liver lipid fractions seems to be

impaired at TCDD doses above 0.1 µg/kg (Cunningham and Williams, 1972), but the significance of this effect has apparently not been explored further.

Evidence of thymic atrophy after TCDD treatment has stimulated more direct study of effects upon the immune responses. Vos and Moore (1974) and Vos et al. (1973) found lymphocytic depletion in the cortex of the thymus and depressed cellular immunity in rats and mice exposed during gestation and the post-natal period. Guinea pigs were similarly affected (Vos et al., 1973). Thigpen et al. (1975) attempted to translate the effect into terms of infectivity and found that doses of 1 µg/kg or more increased mortality and time to death of mice infected with Salmonella bern. Course and severity of disease due to pseudorabies virus was not altered by TCDD. With all the study of TCDD effects, no pattern of pathology, tissue distribution, and biochemical or physiological change has emerged that is consistent enough to suggest a mechanism of lethal effect. McConnell et al. (1977a) quote papers in preparation by Van Logten et al. which show no association of death in TCDD intoxication with adrenal or pituitary hormones or malnutrition.

Pathologic Morphology Resulting From TCDD Intoxication

Much of the more pronounced tissue change resulting from TCDD has been observed following high acute doses of the compound. There is some question whether the acute damage seen represents that observed in a low level longer term experiment or in a field exposure.

Before TCDD as such became an issue Norback and Allen (1969) studied the membrane changes in liver following ingestion of hexachlorohexahydrophenanthrene, a constituent of so-called "toxic fat." The lesions found under electron and light microscopy anticipated the damage later to be

found after TCDD. Most striking was a great increase in the smooth endoplasmic reticulum (SER), with reorganization into a concentric arrangement, and the rough ER became folded and rolled over a period of days to form dense arrays of concentric separate, not spiraling, paired membranes. The authors speculate that SER proliferation in response to intoxication is related to acceptance of enzymes synthesized by the RER. The increased membrane mass may also be a means of sequestering chlorinated hydrocarbon in the lipid phase of the membrane in close proximity to foreign compound metabolizing enzymes. (This paper contains very useful schematic representations of the membrane development sequence.) The same authors later reported similar lesions in rats following administration of 1 μg TCDD/kg/day for 21 days (Norback and Allen, 1973). They found hypoplasia of lymph tissue and bone marrow with progressive anemia and leukopenia. The resultant reduced resistance caused substantial losses due to infection. Monkeys and chickens in the same study were much more sensitive in the latter parameters than rats. They also developed extensive fluid accumulation in all body cavities, probably due to decreased osmotic activity of blood due in turn to serum protein decreases. (Relative sensitivity of chickens has also been noted by Greig et al. (1973).) Monkeys were subject to extensive skin lesions, apparently corresponding to the chloracne of humans, but rats and chickens did not suffer skin damage. Monkeys and chickens also exhibited degeneration of the testes and decreased activity of seminiferous tubules (see also Allen et al., 1975).

Large doses of TCDD (single doses of 50 or 100 μg/kg; 16 to 31 daily doses of 10 μg/kg) to the rat caused severe liver and thymus damage, with icterus and disseminated hemorrhages, especially in the myocardium. The

liver cells increase in size and cellular regenerative attempts occur. Renal tubule cells became vacuolated. No pathological change occurred at doses of 0.1 µg/kg/day over 31 days, at 1.0 µg/kg weekly for 6 weeks or 5 µg/kg in a single dose (Gupta et al., 1973). Fowler et al. (1973) studied the progressive changes in rats over 28 days following a single dose of 5 or 25 µg TCDD/kg. Increased SER, roughly dose responsive, was evident by day three, especially in cells adjacent to biliary drainage. Both RER and SER were greatly increased by day 9, SER was almost at normal levels by day 16, and both were indistinguishable from those of control animals by day 28. Jones and Butler (1974) carried out a similar experiment with rats given a single 200 µg/kg treatment. (A massive dose, considering the minimum effective dose.) Most change was found in centrilobular cells, with degeneration and necrosis of parenchymal cells evident within a week after treatment. They suggest that the multinucleate cells seen in increasing numbers through two months after treatment are formed through membrane disturbance and coalescence of hepatocytes. By 10 weeks some recovery was evident but fibrosis of central veins and sinus dilation still prevailed. No mention of lethality was made, although the dose was on the order of five (female) to ten (male) times the LD_{50}. Longer term treatment of rats (0.001-1.0 µg/kg/day, 5 days weekly x 13) caused almost complete thymus involution at a dose rate of 1.0 µg/kg/day, somewhat decreased cortical thymocyte and lymphoid cell numbers after 0.1 µg/kg/day. Splenic morphology was essentially unchanged at 1.0 µg/kg/day. The latter dose also caused some gross edema, and one of five males had decreased spermatogenic activity. Females at the highest dose tended toward formation of cuboidal epithelium in the uterus and decreased size and numbers of corpora lutea. Ovarian tissue was also abnormal. At all

lower doses no effects were observed (Kociba et al., 1976). Two year continuous feeding studies at TCDD intakes of 0.001 to 0.1 µg/kg/day have just been terminated and histopathologic evaluation is not complete. Gross examination showed that in some rats given 0.1 or 0.01 µg TCDD/kg/day there were increased numbers of nodules in the liver (R.J. Kociba, Dow Chemical Company, preliminary status report; personal communication).

Lesions generally similar to those seen in rats were found in mice given single oral doses up to 200 µg/kg and weekly doses up to 25 µg (Vos et al., 1974). At a dose rate .1 µg/kg/week for 6 weeks decreased thymus weight occurred. At 25 µg/kg/week, serum globulins were decreased, and porphyria and bile duct epithelial proliferation occurred. Similar changes were also reported following treatment of mice with an LD_{50} (30 day) TCDD (McConnell et al., 1977a).

McConnell et al. (1977b) treated rhesus monkeys with heavy single doses of TCDD (in excess of 70 µg/kg) which were fatal in every case. Integumental changes were pronounced, anemia and lymphopenia and an increased neutrophil count followed treatment. Liver, adrenal and kidney appeared to increase in weight relative to body weight, but this effect may have been due to general wasting. With the skin hyperplasia, similar changes occurred in the epithelium of some hollow organs. The major difference from effects in similarly treated rats was the absence of significant liver lesions.

Chronic low level intoxication (500 ppt in diets, about 0.01 µg/kg/day) of monkeys until death at 7-12 months caused anemia and pancytopenia, with extensive hemorrhage. Bone marrow and lymphatic tissues were hypoplastic, and epithelial structures were hyperplastic (Allen et al., 1977).

The pathological changes resulting from absorption of TCDD by horses and cats during the Missouri horse arena episode have been recently described in detail by Kimbrough et al. (1977). The most pronounced and consistent findings were the generalized hepatic fibrosis and necrosis and gastric ulceration. There was an appreciable incidence of tubular degeneration and abscesses in the kidney and capsular thickening of the spleen. Lesions in cats were generally similar.

Effects of TCDD Upon Reproductive Functions

Of the many toxic responses to TCDD, the potential for teratogenicity has received perhaps the greatest notoriety. The early findings of 2,4,5-T teratogenicity raised a considerable public consciousness and the finding that the herbicide was contaminated by high levels of TCDD, also found to cause fetal malformation, focused additional attention on the contaminant. In evaluating this aspect of TCDD hazard it should be noted that the teratogenic potential of a toxicant can be considered in two ways. The absolute teratogenic dose of TCDD to the pregnant animal on a body weight basis is very low, as is true of all TCDD effects. A more realistic way of relating the teratogenic potential, however, is by comparison with the dose required to cause general toxic effects in the mother. For example, in the guinea pig, TCDD teratogenesis has not been studied because the teratogenic dose is apparently higher than the lethal dose.

This section is limited to studies of relatively pure TCDD upon reproductive function in experimental animals. There is reference elsewhere to reproductive studies of 2.4,5-T and silvex, which contain at least some TCDD.

Teratogenic defect resulting from TCDD are rather specific in experimental animals; the principal effects are increased frequency of cleft

palate (reported only in mice) and an abnormality in which the central collection region of the kidney becomes enlarged, with an associated fluid accumulation (Neubert et al., 1973). Limited limb deformation may be seen in a few animals (Sparschu et al., 1971). Intestinal hemorrhage is also seen; this effect is not teratogenic but rather is direct toxic consequence of fetal exposure to TCDD. Fatty degeneration of fetal livers is in the same category (Becker, 1974) as is subcutaneous edema and delayed ossification.

In teratogenicity studies of rodents the usual procedure is to administer intoxicant at some point during, or throughout days 6-15 of pregnancy, which covers the organ forming period. Sparschu et al. (1971) found that the lowest effective dose of 1.25 µg/kg/day caused some fetal mortality, resorptions, and intestinal bleeding. Skeletal abnormalities were limited to delayed ossification. Renal defects tended to occur at about the same frequency regardless of dose. These changes are all a result of direct toxicity to the fetus and are not developmental. Intestinal hemorrhage has been reported in rat fetuses at maternal dose rates of 0.25 µg/kg/day (Khera and Ruddick, 1973). At higher doses up to 8 µg/kg/day the same kind of effects occurred with greater frequency; 0.5 µg/kg/day caused decreased maternal weight gain and higher doses resulted in severe toxicity.

Courtney and Moore (1971) examined rats and three mouse strains and found that a dose of more than 1 µg/kg/day was required to produce kidney defects in rats, and the cleft palate and kidney changes in mice appeared at about 3 µg/kg/day, over days 6-15 of gestation. The C57Bl mouse strain was much more sensitive than the others tested. A somewhat larger group of NMRI mice showed a cleft palate frequency of about 3% (Neubert and

Dillman, 1972), similar to the low frequency groups of Courtney and Moore. The period of maximum sensitivity for TCDD is at the eleventh day of gestation, as distinguished from dexamethasone, for example, which is most effective at day 13 (Neubert et al., 1973).

Continuous administration of 0.001 µg/kg/day to male and female rats for 90 days prior to mating and through weaning of offspring produced no effect on fertility (Murray et al., 1977). The study continued through three generations, with each preceeding generation sacrificed at weaning of its offspring; the third generation was maintained until two years from the beginning of treatment of the f_0 generation. Toxicity and poor litter survival dictated termination of feeding at 0.1 µg/kg/day; 0.01 µg/kg/day caused decreased fertility in generation f_1 and f_2 but not f_0. f_2 and f_3 litters were smaller and growth and survival were decreased after treatment at 0.01 µg/kg/day, but no pathological change was observed in liver, kidney, or thymus.

More recently, Smith et al. (1976) have treated CF-1 mice at doses ranging from 0.001 µg to 3.0 µg/kg/day through days 6-15 of gestation. There was no maternal toxicity at any dose. Cleft palate appeared at 1 µg, renal dilatation appeared at 3 µg/kg/day. No significant effects occurred at 0.1 µg.

A recent report by Courtney (1976) described results of treating CD-1 mice with high doses of TCDD (25-400 µg/kg/day) on days 7-16 of gestation. These very high doses caused no maternal deaths during the gestation period, but resulted in high fetal mortality. The nature of observed terata were typical of TCDD intoxication, but the enormous doses render the study useless for hazard evaluation. However, the

substantially greater incidence of terata after subcutaneous administration, compared with that following oral TCDD suggests that hepatic metabolism may affect TCDD more than has been believed. A dose rate of 25 µg/kg/day orally caused 3% incidence cleft palate; the same dose subcutaneously caused 82% incidence of cleft palate.

There has been little research on effects of TCDD on primate reproduction. Experimental animals are in limited supply and the numbers of offspring are so low that a satisfactory study is almost impossible.

Allen et al. (1977) fed TCDD to eight female rhesus monkeys at a concentration of 500 ppt in the diet, which provided an intake of about 0.3 µg/kg/month. Skin lesions, endocrine and menstrual changes occurred in all of the subjects by 3 months. Six females of the eight were bred after 7 months of treatment; one monkey died before mating and another was excluded from the reproductive study. Three of the animals conceived; two aborted, and one completed a normal pregnancy. Two of the three barren animals were bred four times, the other was bred twice. This dose rate is on the order of 0.01 µg/kg/day and comparison with the rat study of Murray et al. (1977) suggests that reproductive toxicity in monkeys may be somewhat greater than in rats, but is by no means proportional to the lethal dose.

Carcinogenic and Mutagenic Potential of TCDD

Van Miller and Allen (1977) have evaluated pathological changes in rats fed TCDD in dietary concentrations ranging from 1 ppt to 1000 ppb, beginning at animal weights of about 60 g. Estimated weekly intakes of TCDD ranged from 0.0003 µg/kg at a dietary level of 1 ppt to 2 µg/kg at 5 ppb. Actual intake of TCDD at dietary concentrations of 50-1000 ppb was not clear. At 65 weeks the abdominal viscera of surviving animals

were examined surgically and any observed tumors were biopsied. The diet with TCDD was then continued through the 78th week and all surviving animals sacrificed at 95 weeks.

There were 10 animals at each dose range; all those at 50-1000 ppb TCDD died by the fourth week. In the group given 1 ppt there were no tumors, but of those fed 5, 50, 500, 1000, and 5000 ppt 23 of 50 had developed tumors of several types. No control animals for this or a parallel experiment developed neoplasms. The great variety of tumors suggested to the authors the possibility that TCDD is a promoter rather than inducer of neoplastic activity, in view of the usual narrow spectrum of tumors caused by many chemical carcinogens. General pathologic changes have been described in a previous section.

Two studies of carcinogenesis in rats have utilized continuous feeding of TCDD over a two-year period. One program has been completed but the analysis of histopathology and gross tumor incidence have not been completed. The other study, by Dow Chemical Company, has been completed and a preliminary report filed with EPA. Dietary levels were 21, 210, and 2200 ppt TCDD. The maximum dose caused increased tumor incidence in some organs and decreased incidence in others. There was substantial mortality resulting from general toxic effects. The intermediate dose caused moderate systemic toxicity but no tumors and the lowest dose was without effect other than slight liver changes such as induction of increased drug metabolizing capability. These latter changes were seen only in females (J. Davidson, personal communication). The reasons for differences between the Dow and Van Miller studies is not apparent; it is paradoxical that the Dow work had a higher incidence of tumors in control animals, while showing a lower incidence of effect in treated animals.

Cytogenetic studies of TCDD by Green and Moreland (1975) suggested that the dioxin does not have potential for producing chromosomal abnormalities in bone marrow. The dose schedule provided up to 15 µg/kg/daily for 5 days, with sacrifice on day 5. A single injection experiment with sacrifice at four weeks was also negative. Khera and Ruddick (1973) were also unable to find mutagenic activity of 2,4,5-T.

Since it is not possible to obtain an absolutely TCDD-free preparation of 2,4,5-T, the studies of the herbicide may also be of value in considering TCDD effects. Effects of 2,4,5-T and TCDD on dividing African blood lily endosperm cells are not explicitly pertinent to animal studies, but the relation shown between herbicide and contaminant is of interest. Highly purified 2,4,5-T at 10^{-4} M was not effective, but 0.2 µg TCDD/L with or without 2,4,5-T, and commercial 2,4,5-T all caused dramatic mitotic inhibition and chromosomal aberations (Jackson, 1972) (0.2 µg/L = 200 ppt).

Hussain (1972) found that TCDD was somewhat mutagenic using the Ames procedure at doses that were intrinsically very toxic to the organisms. The authors suggested that the limited mutagenic activity was by intercalation of TCDD into DNA. Seiler (1973) has also obtained data suggesting that TCDD is mutagenic in the S. typhimurium TA 1532 strain. There was no indication of the concentrations necessary to produce effects.

Absorption, Distribution, Metabolism, and Excretion of TCDD

The biological action of a foreign compound is highly dependent on its logistics. A chemical must obviously be absorbed from the environment in order to interact, and it must be carried in blood to cells and move

to or across cell membranes to sites of reaction. The agent in its original form may interact with sensitive systems, or it may be converted to an active derivative after entering an organism. Such conversion usually takes place in the liver. A given organ or cell type may have a particular affinity or sensitivity for a toxicant and thereby be singled out for attack. The chemical nature of the agent or its product will also dictate the extent and rate of excretion in urine or bile or respiratory gases. Even with an active excretion mechanism such factors as sensitivity of bladder epithelium or an active enterohepatic circulation may result in toxic effects after elimination seems to have been accomplished.

In spite of the enormous capacity for induction of mixed function oxidases (discussed below) TCDD is metabolized to a very limited extent. Rats given a single dose of 50 µg/TCDD-U-^{14}C/kg by stomach tube excreted about 30% in feces during the two days following treatment, and then 1-2% daily over the 19 days following, to a total of 53%. Urinary-^{14}C was about 13% and respiratory excretion totalled 3.2%. After the initial 2 days, total clearance half-time (T/2) was about 17 days. Residual TCDD at 3, 7, and 21 days was greatest in liver and fat (Piper et al., 1973). The long residence time and limited respiratory and urinary excretion suggested that TCDD was essentially unchanged but this was not verified. As a general rule lipophilic compounds are either sequestered in fat, eliminated as conjugates in bile, or converted to a water soluble form and excreted by kidneys. Absence of urinary excretion therefore implies limited conversion. In a later study of rats given a lower single dose of 1 µg/kg, TCDD was identified in feces but not urine, and again, liver and fat contained the highest concentrations. T/2 was 31 days (Rose et al., 1976). In the same investigation, repeated doses 5 days a week for 7 weeks resulted in some urinary loss, but most TCDD was excreted into feces. In this program

of essentially continuous intake, T/2 was calculated as 24 days. The residual radioactivity in the liver was identified as unchanged TCDD. The report of Vinopal and Casida (1973) is suggestive that TCDD does not metabolize only because after giving a dose of 130 µg TCDD-^{3}H/kg to mice, no mention was made of tritium in the urine. Allen et al. (1975) found that over a 25 day period about 4.5% of a dose of 50 µg TCDD/kg appeared in urine. The daily fraction of total intake excreted gradually increased, which seems indicative of an unusually stable molecule, because at least a modest exchange of tritium with body water should be expected.

The variety of indirect evidence indicating that TCDD is not metabolically altered must be considered in the perspective of other indirect evidence that metabolic change may occur. Beatty and Neal (1975) have shown that pretreatment with phenobarbital decreases and castration increases TCDD toxicity. The former treatment increases and the latter diminishes drug metabolizing capacity. The teratology study of Courtney (1976) also is suggestive that liver metabolism of TCDD occurs. An oral dose of 25 µg/kg/day through the organogenesis period caused only 3% cleft palate among the experimental fetuses, but subcutaneous administration of that dose resulted in 82% cleft palates. In view of the apparent affinity of TCDD for the increased endoplasmic reticulum following induction (Van Miller et al., 1976; Poland et al., 1976), it is possible that induction merely increases the capacity for sequestration of TCDD in a temporarily non-active system, but the remarkable difference seen by Courtney is difficult to ascribe to distribution differences only.

The selective distribution of TCDD into liver and fat has also been shown by Fries and Marrow (1975), Allen et al. (1975), and Van Miller et al. (1976) in rats and by Van Miller et al. (1976) in primates. The

liver TCDD appeared to be sequestered on smooth endoplasmic reticulum (SER), principally that induced by presence of the toxicant (Allen et al., 1975). While liver storage of TCDD in monkeys was much lower than in rats, the difference appeared to be due to the characteristic lesser proliferation of SER in the monkey that has been described by Van Miller et al. (1976). Considering the difference between species in total induced ER membrane, TCDD appeared to bind to endoplasmic reticulum to about the same extent in both species (Van Miller et al., 1976).

Unlike the behavior of other chlorinated hydrocarbons in fasted or underfed animals, TCDD is not lost from adipose tissue with fat mobilization (Allen et al., 1975). In the case of PCB, for example, the fat soluble chlorinated hydrocarbon is liberated with mobilized fat and eventually may reaccumulate in the liver or other target organs. The reason for the difference is not clear, but it seems possible that TCDD may nonspecifically partition into fat, then selectively bind to adipose cell membrane protein. Liver TCDD residues do decrease during fasting, possibly because increased gluconeogenesis makes demands on available labile protein including the endoplasmic reticulum to which the TCDD may be bound. Hepatic subcellular distribution has been further defined by Poland and his associates (Poland and Glover, 1974, 1975; Poland et al., 1976; Nebert et al., 1975); and by Chhabra et al. (1974) in studies in which the genetically determined susceptibility for TCDD induced benzpyrene hydroxylase induction was found to correlate directly with the extent of TCDD binding in liver.

The T/2 for TCDD residence in the body has ranged from about 16-20 days for single dose experiments (Piper et al., 1973; Allen et al., 1975), although Rose et al. (1976) arrived at a figure of 31 days. When TCDD was

administered in the diet over 12 days at dose rates of about 0.5 and 1.5 µg/kg/day, T/2 was found to be 12 days for males and 15 for females (Fries and Marrow, 1975). Rose et al. (1976) calculated a half-time of 24 days after a seven week feeding at dose rates of 1-0.01 µg/kg/day.

The concentration of TCDD reaches a maximum at a given dose rate. Fries and Marrow (1975) found that after 6 weeks of administration retention approached a steady state which would be 10.5 times the daily intake. Rose et al. (1976) found similarly in 7 weeks that a steady state concentration of a little more than 10 times the daily intake could be predicted, agreeing remarkably well with the Fries and Marrow estimate.

The difference in distribution between primates and rats is of interest. Seven days after equivalent doses of TCDD-^{3}H, livers of adult monkeys contained 0.09% of total dose/gm tissue, rats contained 4.54% (Van Miller et al., 1976). In adipose tissue the relationship was monkey, 0.16, rat 3.46, suggesting that storage or retention is not strictly related to binding on inducible membranes. In terms of total body distribution, the rat liver contained 40% of the dose and that of the monkey, 10%. This is a much closer ratio (4/1) than that of liver concentrations (4.54/.09). Skin as a whole stored a much higher percentage of administered TCDD, because of the much higher amount of adipose tissue, in spite of a concentration ratio (3.5/0.16) that was also quite unfavorable.

Whether these differences bear any relation to differences in sensitivity to toxic effects is not known. The difference in liver is at least associated in amount and time with the much greater capacity for induction in the rat. There is no data available to indicate whether the thymus of species like the guinea pig, which is subject to more extensive TCDD induced thymus damage, has a selective affinity for the toxicant as does the

liver in the rat. The thymus of infant monkeys is expected to be highly active, but appeared less retentive compared with other tissues, while the adult rat thymus accumulated more TCDD/unit weight than most organs (Van Miller et al., 1976), further confusing this issue because liver damage is thought to be the primary lesion in the rat.

Each of the single administration experiments utilized heavy doses of TCDD, taking advantage of the long delay in onset of symptoms and death to obtain improvement in radiochemical detection capability. Studies in which 400 µg/kg (about 20 x LD_{50}) were administered (Van Miller et al., 1976) produced liver concentrations of about 3.6 ppm. Kociba et al. (1976) caused discernable but minor hepatic lesions at a total dose of 0.65 µg/kg given over seven weeks. The expected concentration of TCDD in liver at this intake is on the order of 20 ppb (Rose et al., 1976). A ten-fold lower dose caused no histopathologic damage. It is not surprising therefore that the beach mice studied by Young et al. (1974) which accumulated 300-500 ppt TCDD in their livers, did not show liver morphologic lesions.

Behavior of TCDD in the Physical Environment and in Submammalian Species

The extremely high toxicity of TCDD and the very small amounts of the agent present in 2,4,5-T and in the environment present a problem that is different less in philosophy than in scale. There is a tendency on the one hand to dismiss TCDD as a pollutant because the amount available at any one point or even sector is minute, and on the other to assume that any amount in excess of true zero residue is catastrophic, because of the enormous toxicity of the material. The amount of TCDD distributed on vegetation after a typical treatment is so small as to defy direct analysis and the quest for residues in biological materials has forced analytical

sensitivity closer to zero than has been the case with any other pollutant. In spite of these unique characteristics, TCDD must be considered in the same way as any other chemical: How much is present in the human environment? How persistent is it? Can it move to unintended targets? If so, will the amount assimilated by humans or other organisms reach harmful proportions?

Present manufacture of 2,4,5-T contains about 0.02 parts TCDD per million parts 2,4,5-T, although manufacturers claim only about 0.05 ppm. Some lots contain only about 0.01 ppm. A two pound per acre application of 2,4,5-T will, therefore, distribute a little less than 20 µg TCDD/acre. It is necessary to predict what may be expected to happen to that material by examining existing laboratory data.

The environmental persistence of TCDD is highly dependent on the conditions to which it is exposed. When mixed into soil, degradation was found to be very slow, with a half time of one year (Kearney, 1972). Because the material was introduced as an acetone solution there has been some question about possible crystallization and limited access of TCDD to soil organisms. However, TCDD in 2,4,5-T deposited on leaves and exposed to direct sunlight was found to degrade with a half-time of a little more than an hour (Crosby et al., 1977). The latter study shed a good deal of light on earlier studies in which pure TCDD was carefully coated on clean glass slides, water, or dry sterile soil for exposure to sunlight, and found to be almost insensitive to photodecomposition (Plimmer et al., 1973; Kearney et al., 1972). Crosby et al. noted that reduction would proceed in methanol, and that the reaction slowed significantly in highly purified reagent. The finding that benzene accelerated the degradation of TCDD in aqueous systems (Plimmer et al., 1973) suggested that a

good hydrogen donor was essential to photodecomposition. This conclusion has been verified in more recent work (Crosby et al., 1977). In that study TCDD with 2,4,5-T was also applied on soil, and a half-time of 50-55 hours could be inferred from the data. This finding provides some suggestion about the reaction time in indirect light, because the rough surface will provide shaded areas where reaction must be slower. As yet, studies of the effect of various parts of the spectrum emerging from light transmitted through leaves, or reflected from various surfaces has not been done. The absorption maximum for TCDD is known (Crosby et al., 1973) but the relative sensitivity to degradation at that wave length apparently is not.

Accumulation of TCDD in the biota has been studied both in the laboratory and in the field, but there are still many gaps in the needed data.

In spite of the limited solubility of TCDD (0.2 ppb), it can accumulate in aquatic organisms to the extent it is available on sediments or other reservoir (Matsumura and Benezet, 1973). In their experiments, TCDD was deposited on sand, in solvent which was evaporated to leave the dioxin as a film on sand particles. The sand was placed in a small confined static aqueous system and various aquatic organisms added. Brine shrimp reached a concentration of 157 ppb, mosquito larvae concentrated TCDD to 4150 ppb and silverside fish accumulated virtually no TCDD. It may be assumed that the concentration of TCDD in water was maintained throughout the experiment by the excess dioxin residue on sand.

When sufficient TCDD to represent 162 ppb in the aquarium was ingested by algae which were then placed in an aquarium with Daphnia and with Ostracods, the latter species accumulated TCDD to concentrations of 879 and 279 ppb, respectively. They also showed that mosquite fish concentrate

TCDD to a lesser extent than do the bottom feeding larvae on which they feed. It is clear that accumulation does occur, but depends on maintaining saturation of the ambient water.

Matsumura and Benezet (1975) also examined movement of TCDD which had been bound to sand, to a sandy loam soil (inorganic to organic soil). Very little TCDD was translocated by a slow leaching with water. Subsequently, a more complex food chain system of soil, water, algae, duckweed, snails, daphnids, gambusia, and catfish was assembled (Isensee and Jones, 1975). ^{14}C-TCDD bound to soil in concentrations varying from 0.0001 to 7.45 ppm was placed in aquaria. The residues found in the various species after 33 days exposure tended to peak at the Daphnia stage. Gambusia were added at the end of the exposure period with access to water and other organisms for 3 days. Catfish fingerlings were exposed for 6 days, beginning after all other organisms were removed. The catfish accumulated TCDD to roughly the concentration found in the first biological stage, the algae. Nonetheless, at a water concentration of 7 ppt TCDD a terminal accumulation ratio of more than 10,000 was found in catfish, representing TCDD concentrations of about 100 ppb (Isensee and Jones, 1975). The work also shows, however, that as soil or water concentrations decrease, so also do concentrations in the organisms of the system. Toxicity was not studied although the higher tissue accumulations exceeded mammalian toxic doses, assuming uniform distribution of dosage. The authors point out that with the long latency of TCDD toxicity, the fish may not have had time to develop lesions and die.

Ward (1976) examined the persistence of TCDD in lake water and sediments. As expected, TCDD is almost entirely bound to lake sediments and is subject to very limited but nonetheless real metabolic degradation when microorganisms were present. The slow microbial activity is enhanced

by presence of nutrients. Ward suggested that water-mediated evaporation of TCDD occurred to a limited extent.

Toxicity of TCDD to coho salmon has been assayed by Miller et al. (1973). TCDD was administered in the diet and dosage expressed as ng TCDD per gram organism mass. At 13.1 ng/g only about 30% of the experimental group survived for 80 days past exposure, and at 5.4 ng/g almost half the fish did not survive. Deaths often did not occur until 10 days after the end of exposure, and some lethally affected fish survived 60 days or more. In studies of rainbow trout there were no apparent effects at intakes of 6.3 ng TCDD/g or greater. Mosquito larvae pupated at a normal rate in water containing 200 ppt TCDD; snails reproduced normally and oligochaete worms suffered some reproductive deficit at the same concentration. Beatty et al. (1976) found Rana catesbiana tadpoles to be remarkably resistant, with no apparent lethal effect by intraperitoneal doses of 1000 µg TCDD/kg. Doses up to 500 µg/kg did not affect adult frogs, and no histopathological lesions appeared.

Field studies of fish and mammals have provided evidence both supporting and contradicting claims of TCDD accumulation in higher animals. An evaluation of biota in the area used by USAF for training bomber crews in application of military defoliants showed little accumulation of TCDD in fish living in the streams of the area. The area was literally saturated with 2,4,5-T, receiving on the order of 1000 lb/acre during the peak year, containing up to 2-10 ppm TCDD. Mice in the area accumulated tissue burdens of several hundred ppt as measured at the end of the spray program, when the sandy soil had accumulated residues up to 700 ppt. These animals demonstrated no pathological changes (Young et al., 1974), which is not surprising when extrapolating the data of Rose et al.

(1976) and Kociba et al. (1976) to relate tissue levels and effective dose rates in rats. Given the extended period of exposure, it seems possible that the mouse population could adapt through survival of non-responding strains over the estimated 30 generations of exposures. It may be also that fish strains have evolved similarly as non-accumulators of TCDD, although such adaptation seems unlikely.

Fish have also been analyzed in drainages from areas in Texas and Arkansas which had been extensively treated with 2,4,5-T (Shadoff et al., 1977). The Texas samples were from a pond which collected run-off from a watershed on which the herbicide was used for brush control. The Arkansas site was a pond into which adjacent rice fields were drained and from which irrigation water was obtained, in essence recycling any contaminants. Treatment was 1.25 lb 2,4,5-T/acre, 4-8 weeks prior to flooding. The cycle had been in use for 18 years. Fish from the Texas site did not contain TCDD, at detection limits of 5-7 ppt. Mud at a detection limit of 3 ppt and water at 0.1 ppt were also negative. Six human milk samples obtained from the San Angelo area near the Texas site were also negative at a detection limit of 3 ppt. No TCDD was found in the Arkansas fish samples. Meselson and O'Keefe (correspondence to Representative James Weaver, 1977) have presented preliminary findings of about 1-2 ppt TCDD in human milk from the San Angelo area, and from an area in western Oregon.

Cows milk samples from 2,4,5-T-treated areas of Oklahoma, Arkansas, and Missouri were found to be negative for TCDD by Mahle et al. (1977) with detection limits on the order of 1 ppt. TCDD in beef fat was undetectable in two series of animals which had grazed on 2,4,5-T treated pastures in Oklahoma, Texas, and Missouri. In a third group of seven

animals confined in an entirely sprayed pasture, three samples were positive at the detection limit of 3-4 ppt. (Kocher et al., 1977). TCDD has been found, however, in several beef cattle maintained in a field experiment on fields which had received 1, 2, 3, or 4 lbs 2,4,5-T/acre. Average TCDD in fat was 20.3 ppt in the 4 lb/acre group, but there were only three samples, one of which was extremely high. At 3 lb/acre the average was 12.8 ppt, again derived from some high values and many negatives. Interpretation of this data by EPA is still in process, and is subject to some argument.

A number of wild animal samples were obtained in treated forest areas of western Oregon, several of which contained unusually high concentrations of TCDD. The unique character of the data raises some questions, since there seems to be few or no samples containing TCDD concentrations intermediate between the low background level and the 100-200 ppt values in a few animals.

An issue has been raised about formation of TCDD during combustion of 2,4,5-T, and subsequent distribution in air. Stehl and Lamparski (1977) ignited grass treated with 2,4,5-T in a system where combustion was self supported and found less than 0.0002% converted to TCDD. Combustion of 2,4,5-T and trichlorophenol on filter paper led to production of even less TCDD. If the conversion of a field application were at this rate, about 2 parts of available 2,4,5-T per million would be converted, which is considerably more than the residual contaminant level. It should be remembered, however, that burning would not be done until foliage dries, and with a 1-2 week half time it is doubtful whether much herbicide would be available for conversion. Even this maximum level of conversion is probably not of great concern, since the product would be diluted with combustion gases and air in millions of cubic meters of atmosphere. The

effect of exposure to light on decomposition while in the atmosphere is unknown. Masking or wavelength shifts may have an inhibitory influence on photodegradation, but the duration of atmospheric suspension and light exposure should enhance the reaction.

Induction of Microsomal Enzymes

TCDD has been found to be a remarkably potent inducer of many enzymes which act on foreign chemicals. The effect is apparently dependent upon synthesis of additional enzyme, rather than activation of partially synthesized or otherwise inactive complete protein. TCDD also increases activity of δ-aminolevulinic acid synthetase in some species, resulting in an accumulation of porphyrins in tissues and excretory routes. The potency of TCDD for inducing some enzymes is between four and five orders of magnitude greater than that of such classical inducing agents as 3-methyl cholanthrene or phenobarbital. According to Hook et al. (1975a) TCDD induces cytochrome P-450 at doses of fewer molecules of TCDD than there are of P-450 in the liver. This property is important because the induced enzymes may increase conversion of other organic intoxicants to non-toxic products, or conversely may increase conversion of non-toxic materials to highly reactive intermediates which exert profound toxic effects. Many carcinogens are activated in this way. The changes in "drug-metabolizing" enzymes also have been used to describe profound genetic differences among animal strains, and their respective response to intoxication.

Enzyme induction may also serve as a predictor of general toxicity, since the induction potency seems well correlated with lethal, acnegenic, and teratologic potential (Schwetz et al., 1973; Poland and Glover, 1973a).

The enzymes which oxidize foreign chemicals have evolved in response to reactive groupings on molecules found in nature. While natural and synthetic chemicals may represent an almost infinite variety of structures, the building blocks are the same, and they exist in relatively limited numbers. Consequently, organisms have not had to develop an infinite number of enzymes to survive, but rather have developed enzymes specific for reactive groups.

The effects on these systems are among the more sensitive changes found to be caused by TCDD. To simplify presentation the following table identifies specific enzymes and the TCDD doses at which responses have been observed. Unfortunately, few explorations of the entire dose response curve have been made; the "no observed effect" intake has usually not been determined, nor has there been an adequate study of the impact of low level chronic studies on the microsomal enzymes. Most of the data in the table refers to rats. Relatively few studies of mice have been made, and in the guinea pig the effective doses approach the lethal dose, and observed changes are minor. Hook et al. (1975b) administered 0.175 μg/kg to guinea pigs and found a slight increase in liver biphenyl-4-hydroxylase and biphenyl-2-hydroxylase, and no change in aryl hydrocarbon hydroxylase or UDP glucuronyl transferase.

Several generalizations appear applicable to the enzyme inductive properties of TCDD. While most microsomal enzymes are increased after TCDD, the enzymes identified as demethylating aminopyrine, benzphetamine and morphine are decreased (Lucier et al., 1973).

Females seem to be more sensitive to the inducing effects of TCDD (Lucier et al., 1975b). While effects on UDP glucuronyl transferases are substantial, steroid glucuronyl transferases are not changed. The

Induction of Foreign Compound Metabolizing Enzymes by TCDD (1)

Enzyme	TCDD Dose (μg/kg)	Extent of Initial Induction (x normal)	Duration of Increased Activity	References	Remarks (liver unless other tissue specified)
p-Nitrophenol-UDP glucuronyl transferase	3	14	>34d	Lucier et al., 1975a	pregnant rats (2)
	3	7	>34d	Lucier et al., 1975a	neonate, (3)
	25	6	73d	Lucier et al., 1975b	male rat
	5	5	>28d	Hook et al., 1975b	
	0.2	2.5	--	Lucier et al., 1973	
				Lucier et al., 1973	
Benzpyrene (aryl hydrocarbon) hydroxylase	3	14	>21d	Lucier et al., 1975a	pregnant rats (2)
	3	2		Lucier et al., 1975a	neonate, (4)
	2.5	14	--	Berry et al., 1976	pregnant rat, (5)
	2.5	100	--	Berry et al., 1976	fetal liver
	0.2	7	--	Lucier, 1973	male rats (6)
	0.2	8	--	Hook et al., 1975b	female rats (7)
	1.0	1.5	--	Hook et al., 1975b	male (7)
	25	3	--	Hook et al., 1975b	male rat liver
	25	>70	--	Hook et al., 1975b	male rat kidney (8)
	10	180	--	Poland and Glover, 1974	rat kidney
	10	10	--	Poland and Glover, 1974	rat lung
	10	100	--	Poland and Glover, 1974	rat intestine
	.08	3	--	Poland and Glover, 1974	C3H/HeN mouse
NADPH diaphorase (dehydrogenase)	90	13	>15d	Beatty and Neal, 1976	male rat liver (9)
Aniline hydroxylase	5	2	>15d	Lucier et al., 1973	
	0.2	1.2	--	Lucier et al., 1973	
Cytochrome P-450	1.0	1.6	--	Lucier et al., 1973	male, 3 day
Cytochrome B_5	1.0	1.4	--	Lucier, 1977	male, 3 day
Biphenyl-2-hydroxylase	25	13	--	Hook et al., 1975b	rat liver
	25	18		Hook et al., 1975b	rat kidney (11)

Induction of Foreign Compound Metabolizing Enzymes by TCDD (cont)

Enzyme	TCDD Dose (µg/kg)	Extent of Initial Induction (x normal)	Duration of Increased Activity	References	Remarks (liver unless other tissue specified)
Biphenyl-4-hydroxylase	25	2		Hook et al., 1975b	rat liver
	25	40		Hook et al., 1975b	rat kidney
Amino levulinic acid synthetase	25	none	--	Woods, 1973	
	25	2		Goldstein, 1973	mouse (11)
	1.5 ng/egg	2		Poland and Glover, 1973c	chick embryo

[1]This table is selective and is intended to show the variety of work done, the dose ranges studied, and duration of effects. In most cases the lowest dose producing effect has been shown and data for higher doses omitted. The references noted should be consulted for greater detail.

[2]Pregnant rats, treated on day 10 of 23 day gestation, sacrificed 21 days post partum.

[3]Mother treated at day 5 of gestation; activity measured at day 21 postnatal. No increase in activity before parturition.

[4]Same as 2 except measured at day 8 postnatal.

[5]TCDD at day 17 of gestation, sacrificed at day 20.

[6]Male rats, sacrificed 3 days after TCDD.

[7]Male rats did not respond at this dose; there was a small but significant ($P < 0.05$) increase in amino pyrine demethylase and cytochrome P-450 in females at 0.2 µg TCDD/kg.

[8]Resting activity below detection limit.

[9]Maximal activity in cytosol at 7 days, still rising in microsomes at 15 days.

[10]5 µg/kg produced no effect.

[11]Resting activity very low in these tissues; factor of increase approximate.

bulk of induction occurs in liver but a similar but usually lesser increase can occur in the kidney and some other tissues (see table). In kidney, activity seems concentrated in the "outer stripe" of the medulla and in the cortex. This region contains the terminal straight segments of the proximal tubules, which appear to be the only significantly inducible cells in the kidney (Fowler et al., 1977).

The induction of aryl hydrocarbon hydroxylase (AHH) by TCDD has been very useful in explaining genetic variability in receptor and effector mechanisms. TCDD is about 3×10^4 times more potent than 3-methylcholanthrene, producing half maximal stimulation in the rat liver at 0.85 nmoles/kg and in the C3H/HeN mouse (female) at a dose of 0.42 nmoles TCDD per kg (Poland and Glover, 1974). While the susceptibility to AHH induction is about the same in the species tested (ED_5 = 0.4-1.2 nmole/kg), and the LD_{50}s differ by about two orders of magnitude, this difference does not detract from the value of the system in studying the genetics of drug response and the great problems of non-homogeneity of exposed populations.

There is known to be a profound difference in the responses of various strains of mice to AHH induction by 3-methylcholanthrene (3-MC), leading to a designation of "responsive" and "non-responsive" strains. Even at high doses, 3-MC fails to cause induction in the non-responsive animals. TCDD, however, will elicit an amplified enzyme activity in such mice (Poland and Glover, 1975; Chhabra et al., 1974).

Poland and Glover (1975) and Neubert et al. (1975) have shown that the genetically non-responsive mice have the genetic apparatus necessary for expression of the inducible activities and suggest that the difference arises from a mutant form of inducer-binding receptor with a diminished affinity for aromatic hydrocarbons. The induction process is apparently

an increase in de novo synthesis of enzyme, rather than activation of preexisting protein or decreased degradation of protein (Haugen et al., 1976). Whether the effect of TCDD on inducible enzymes has implications on other aspects of protein synthesis can only be speculated upon. Poland and Glover (1975) showed about a 10-fold difference in the dose necessary to cause induction. Through the finding that heterozygous offspring of C57Bl/6J (responsive) and DBA/2J (non-responsive) strains are intermediate in sensitivity, they support the contention of Poland and Glover (1975) that the difference is a receptor site mutation. Later studies have strengthened that premise in finding that hepatic accumulation of ^{14}C-TCDD administered intraperitoneally was greater in responsive than non-responsive strains (Poland et al., 1976). In the same study, in vitro examination of binding in the soluble fraction of liver cells showed that specific binding sites exist, and that their affinity correlates with the genetic capability for induction. Furthermore, tests of an extended series of halogenated dioxins and dibenzofurans showed that cytosolic binding corresponded closely with the induction potency of the chemical.

Niwa et al. (1975) have measured AHH induction by TCDD in a variety of cell cultures. They used 10 established cell lines, human lymphocytes, and primary fetal cultures from chick, rat, rabbit, hamster, and four strains of mice. Generally, kinetics of TCDD induction in each cell type is similar to that of 3-MC, and the induction was inhibited by actinomycin-D and cyclohexamide, which are inhibitors of protein synthesis. They found no relation between inducibility and cytotoxicity of TCDD, which is further evidence that TCDD itself is not metabolized. The sensitivity of some cell lines is such that the authors suggest use

of H-4-II-E cells (derived from Reuber hepatoma H-35 in rats) as a bioassay, with a suggested sensitivity below 1 pMole (∿ 0.0003 μg) in 3 ml of culture medium.

The same kind of genetic differences have been studied in human lymphocyte cultures, which represent the genetic background of the cell donor and are therefore useful in making relatively non-invasive studies of potential human response to intoxication. The sensitivity to TCDD induction of AHH is about 50-fold greater than to 3-MC induction, considerably less than the 30,000-fold difference in responsive mice (Kouri et al., 1974). Atlas et al. (1976) have carried these studies forward with lymphocytes from human tissues, but have as yet dealt only with 3-MC, not TCDD. Part of the stimulus for examining human cells lies in the suggestion that extent of induction at contact sites (i.e., skin, lung) may relate to the probability of cancer initiation by activated carcinogens (Kellerman et al., 1973). Although TCDD is as yet not established as a carcinogen such research may provide suggestions about the extent of expected variations in other TCDD responses in humans.

2,4,5-T

General Toxicity in Laboratory Animals

Apparently the first published account of 2,4,5-T acute toxicity was that of Drill and Hiratzka (1953). The LD_{50} for dogs was about 100 mg/kg; the principal symptom was a mild incoordination. When fed five days weekly for 90 days, doses up to 10 mg/kg/daily were without evident effect, but 20 mg/kg/day was lethal to all 4 treated animals between 11 and 75 days after the first dose. Symptoms were limited to muscle twitching and impaired swallowing. A field study by Grigsby and Farwell

(1950) [as quoted by Rowe and Hymas (1954)] exposed a variety of stock on pasture immediately after spraying 2,4,5-T at 2-4 times usual levels, with no effect.

Rowe and Hymas (1954) summarized the available data at that time and estimated the acute oral median lethal dose for male rats to be about 500 mg/kg, male mice 389 mg/kg, and guinea pigs 381 mg/kg. LD_{50}s for various esters were higher than for the acid. A sheep fed the propyl glycol butyl ester of 2,4,5-T died after 369 daily doses of 100 mg/kg and another sheep and a cow died after seven daily doses of 250 mg/kg (Palmer and Radeleff, 1964). The triethylamine salt of 2,4,5-T at 100 mg/kg caused no observable effect after 481 days of treatment at 100 mg daily. As single animal observations these can be considered rough estimates only, but they convey the generally high doses necessary to cause harm in ruminants. 2,4,5-T has been shown to cause decreased volatile fatty acid production in vitro at concentrations of 500 µg/ml or greater (Kutches et al., 1970); this concentration is probably greater than can be maintained by a survivable daily dose of 2,4,5-T.

Subchronic (90 day) feeding of male and female rats caused no effects below 30 mg/kg/day, but body weight and food intake were depressed after treatment at 100 mg/kg/day. Alkaline phosphatase and SGPT were elevated and erythrocyte count and hemoglobin was decreased. The histopathologic findings were limited and inconsistent (McCollister and Kociba, 1970). Rats were able to tolerate 186 mg/kg/day treatment with mixed mono-, di-, and tripropylene glycol butyl ether esters of 2,4,5-T over 90 days but developed some indications of toxicity. At a dose of 18.6 mg/kg no evidence of toxicity was observed (Dow Chemical Company, 1961).

A two year study of rats given 3 to 30 mg 2,4,5-T/kg/day has very recently been completed by Dow Chemical Company. Much of the analysis has yet to be completed, including morphologic pathology. Animals that received 30 mg 2,4,5-T/kg/day were found to have increased urinary porphyrin excretion after 4 months of treatment, and this change continued through the entire 2 year period. No changes were found in any other hematologic, urinary, or clinical chemistry measurement at that rate of intake, and the increased porphyrin excretion did not occur at 10 or 3 mg/kg/day (R.J. Kociba, Dow Chemical Company, preliminary status report; personal communication).

A feeding study of reindeer was prompted by allegations that a high incidence of death and abortion occurred in 1970 in a herd after use of phenoxy herbicides the previous year. Fifteen of thirty pregnant reindeer were fed birch leaves which had been sprayed with a 2,4-D/2,4,5-T mixture, through a 1.5 month period late in gestation. The daily dose of phenoxy acid was about 1 mg/kg/day. No clinical chemical changes could be detected, nor did any changes appear at autopsy either in the adult animal or the full term fetus (Erne, 1976).

The toxic effects of 2,4,5-T in rodents have been studied in much more detail than the gross toxicity studies already described. Highman et al. (1976a, 1976b) established that lethal doses in pregnant or nonpregnant mice caused myocardial lesions, bone marrow aplasia, and lymphocytic depletion in thumus, spleen, and lymph nodes, and that animals which remained apparently healthy despite similar doses did not suffer the same severity of lesions. A mild hemolytic anemia did occur in such animals. Pregnancy did not augment the toxic effect. It was also clear that major differences exist in the response of specific strains. Many NCTR mice were

seriously affected at doses below 60 mg 2,4,5-T/kg/day (6-9 days of treatment while most CRBL mice remained unaffected at doses as high as 90-120 mg/kg/day at the same time. The histopathology, hematology, and blood chemistry changes in treated mice were also studied. In many monitored animals, some myocardial fibers were found to be pale and swollen, with longitudinal striations. The condition was rarely seen in treated apparently non-intoxicated animals. Necrotic changes were also often seen in the outer myocardium. High doses caused thymic atrophy, with almost no lymphocytes in the cortex and increased numbers in the medulla (this effect was enhanced in late pregnancy by the normal tendency to cortical involution in pregnant mice). Spleens were often atrophied, and thyroid follicles were enlarged with enlarged epithelial cell. In the liver, glycogen was often depleted. Blood chemistry changes appeared not to be marked.

2,4,5-T has been found to cause increased liver weight in rats at doses of 167-334 mg/kg over a two-day period, apparently through stimulation of protein and RNA synthesis, but not as newly formed 2,4,5-T metabolizing enzymes. The change reverses after withdrawal (Chang et al., 1974; Rip and Cherry, 1976). Liver nuclei isolated from treated animals were more active in RNA synthesis than those from controls. 2,4,5-T at a concentration of 4 mmolar is capable of sharply inhibiting in vitro incorporation of mevalonate-^{14}C into non-saponifiable lipids by rat liver (Olson et al., 1974). If data obtained in mice by Nony et al. (1976) can be applied, this concentration is approachable at single doses of 50 to 100 mg/kg, which are survivable by most species.

Koschier and Berndt (1976a, 1976b, 1976c) have described the effect of 2,4,5-T upon renal physiology. Large doses of 2,4,5-T appear to

impair secretion of itself by decreasing the activity of the organic acid transport system in the proximal tubule. Organic base transport was also depressed. Small doses of 2,4,5-T were excreted very rapidly, but large daily doses led to renal depression and retention of the compound. The authors consider the data to support the conclusion that transport of phenoxy herbicides is an active process.

The psychopharmacology of 2,4,5-T does not seem to be a popular field of study. In the one study found, single doses of up to 100 mg/kg were given on day 7, 8, or 9 of pregnancy. The male offspring of females given the highest dose exhibited more exploratory open field behavior, but no difference was found in females. The highest doses caused decreased litter size but no increase in malformations (Sjödén and Söderberg, 1972).

Poultry seem relatively insensitive to 2,4,5-T. Whitehead and Pettigrew (1972) found that a single oral dose of 900 mg/kg to 4 week old chicks caused 40% lethality. However, feeding of 1000 mg 2,4,5-T per kg diet/day for three weeks to chicks, beginning at one day of age caused only some slowing of growth; 5000 mg/kg diet was lethal. At levels not causing gross toxicity, plasma calcium and magnesium were not affected. Given a choice, the birds rejected the treated diet in favor of non-contaminated food. Turkeys fed 2,4,5-T at a rate equivalent to 62 mg 2,4,5-T acid daily for 11 days were unaffected (Roberts and Rogers, 1957). Bjorklund and Erne (1971) introduced various 2,4,5-T derivatives into water and feed of chickens, quail, pheasants, and ducks. In water the LC_{100} of the triethanolamine salt for chickens over a 29 week period was 1000 ppm acid equiv. (about 200 mg/kg). In the diet of other species over

a 7-day period, the LC_{50} was in excess of 2000 ppm. Kenaga (1975) has extensively reviewed avian toxicity and safety of birds in areas treated with herbicides and concludes that the no observed effect levels are substantially above amounts that might be contacted in a field application.

Effects of 2,4,5-T on Reproduction

The teratogenic potential of 2,4,5-T has received wide publicity since 1969 when allegations were made that its use as a military defoliant had caused fetal malformations in the Vietnamese population. A succession of studies over the next three years confirmed the teratogenicity of 2,4,5-T, but the implication of the herbicide in any increase in birth defects has not been supported. The initial report of 2,4,5-T teratogenic effect (Courtney et al., 1970; Bionetics Research Laboratories, 1970) described cleft palate and cystic kidneys at doses of 46 and 113 mg/kg/day on days 6 through 14 of gestation. Of the two lesions, cystic kidney appeared to be a more sensitive indicator. The 2,4,5-T used in the study was found to contain 30 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and the respective toxicities of the two agents were still unclear at that time. The data was also criticized for an unusually high and variable incidence of embryotoxicity in control animals (Neubert and Dillman, 1972).

Emerson et al. (1971) evaluated a commercial 2,4,5-T with less than 0.5 ppm TCDD and found that 24 and 40 mg/kg/day through days 6-15 of gestation caused no teratogenesis in rats. Sparschu et al. (1971) then found that 50 mg was also nonteratogenic, although there was a slight increase in incidence of delayed skull ossification. (Such alteration in development is not considered teratologic, because the abnormality disappears with age. One criterion of teratogenic effect is irreversibility.)

Treatment at 100 mg/day for days 6-10 caused generalized maternal toxicity, with only 4 survivors of 25 treated animals. Intestinal hemorrhage, considered a common fetotoxic but not teratologic lesion, was found in only one pup in this study. Khera and McKinley (1972) found a similar dose response.

In mice the effective dose is similar to that in rats. At 50 mg 2,4,5-T/kg/day, through days 6-15 of gestation, the frequency of cleft palates was increased from 4.7% to 20% and resorption frequency increased. The incidence was raised to 73% by a dose of 110 mg/kg/day with a decrease in fetal weight (Bäge et al., 1973). The high dose also caused a two-fold increase in rib and vertebral malformation, but only marginal increase in dilated renal pelvis. Neubert and Dillman (1972) found increased cleft palate in NMRI mice at doses over 200 mg/kg/day through days 6-15, increased embryo lethality at doses over 45 mg/kg/day reduction in fetal weight above 15 mg/kg/day. Strain differences in mice are significant. The dose causing at least one cleft palate in 50% of litters was 25 mg/kg/day in the most sensitive of 5 strains tested and 105 mg/kg/day in the least sensitive (Gaines et al., 1975). Roll (1971) found detectable teratogenesis at doses above 35 mg/kg/day through days 6-15 of pregnancy. The no-effect level with respect to teratogenesis was established as 20 mg/kg/day.

Hamsters are less sensitive to cleft palate, but do have a somewhat higher incidence of delayed head ossification which is not generally defined as a teratologic manifestation (Collins and Williams, 1971). The doses ranged from 40-100 mg/kg and there was a considerable difference among samples of 2,4,5-T of different sources and levels of TCDD contamination.

Fetal rabbits are apparently unaffected by maternal doses up to 40 mg 2,4,5-T/kg/day through days 6-18.

Ruminants are apparently also quite resistant to teratogenic effects of 2,4,5-T. Binns and Balls (1971) fed 100 mg 2,4,5-T/kg to 11 ewes from the 14th to 36th day of gestation, and fed 100 mg 2,4,5-T(PGBE)kg to another group during the same period. No evidence of deformity appeared in any of the lambs. The TCDD content was 1 ppm.

The no apparent effect levels in rodents reported in the preceeding studies contrast sharply with data in abstracts of reports by Konstantinova (1974a, 1974b), who is said to have found that 4.2 mg/kg/day of the butyl ester of 2,4,5-T through the entire gestation period caused embryotoxicity, nervous and hematologic change and histopathology in the mother. A dose rate of 0.42 mg/kg affected growth and development, nervous, liver and kidney functions and lowered fertility. The succeeding generation was found to have minor changes in organ weights. (Direct translations of these papers are not available; data quoted is obtained from Chemical Abstracts.)

There has been limited study of the teratogenic effect of 2,4,5-T in primates. Dougherty et al. (1975) administered 0.5, 1.0, and 10 mg/kg/day to groups of 10 pregnant rhesus monkeys from day 22 through 38 of gestation. The high dose was established after finding that 12 mg or more 2,4,5-T/kg daily for 18 days caused vomiting and weight loss in monkeys of both sexes. No teratogenesis occurred, although there were 1 or 2 abortions, premature births, or neonatal deaths in all groups, including the control.

The importance of TCDD in the teratogenicity of 2,4,5-T was examined soon after the contamination was recognized. Courtney and Moore (1971) tested 2,4,5-T with 0.5 and 0.05 ppm TCDD, 2,4,5-T and TCDD together and

TCDD alone in mice. It was evident that the concentrations used or addition of 1 μg TCDD/kg/day through days 6-15 did not alter the apparent teratogenicity of 2,4,5-T. Neubert and Dillman (1972) reached a similar conclusion, suggesting that to potentiate 2,4,5-T effect on pregnant mice at least 1.5 ppm TCDD is necessary. Collins and Williams (1971) observed additive effects of 2.9 and 45 ppm TCDD contamination of 2,4,5-T administered to hamsters, but many of the changes appeared characteristic of primary TCDD intoxication.

Nutritional status seems to have little influence on the effect of 2,4,5-T on reproductive functions. Hall (1972) fed 250 and 1000 ppm 2,4,5-T with diets containing 20% and 60% casein. Resorption and stillbirth incidence and litter size were similar in all groups, although fetal organ and placenta weights were decreased in the high 2,4,5-T, high protein group. Effects of low protein intake were augmented by the high concentration of herbicide.

Incubating eggs have been considered to be particularly vulnerable to herbicides because they remain in one place, and because the eggshell is porous. A number of studies of 2,4-D effect on eggs have appeared since 1967, with conflicting results (see section on 2,4-D), but 2,4,5-T has not received much attention. Somers et al. (1973) sprayed hen eggs with a formulation containing 2,4,5-T and 2,4-D in a treatment equivalent to 11.2 kg/hectare (10 times normal field application rates) prior to incubation without effect on hatchability or early survivability of chicks. They then treated eggs of the pheasant, as a genetically more heterogeneous species, in the same way, without adverse effect. Entry of herbicide into the egg was verified analytically. The treatment did, however, increase weight gain in male chicks during the first four weeks of life (Somers et al.,

1974). The LD_{50} of 2,4,5-T in DMSO, injected directly into the air space of chicken eggs at doses up to 125 mg/kg has been calculated to be 62 mg/kg, and in acetone carrier, 133 mg/kg (Strange et al., 1976). No teratogenic effect was evident. The solvent toxicity was shown to be substantial, but it seems clear that acquisition of an effective dose of 2,4,5-T by an egg in the field is highly unlikely.

In fact, immersion of hen eggs in 1% 2,4,5-T was ineffective, and a 5% solution was only moderately effective. The eggs were immersed for 10 seconds, then returned to incubation (Gyrd-Hansen and Dalgaard-Mikkelsen, 1974).

Insects may be more sensitive; Dävring and Sunner (1971) and Dävring (1975) have shown that while Drosophila are highly resistant to lethal effects of 2,4,5-T ester (LD_{50} = 4700 ppm in the diet), 1 ppm caused disturbed egg follicle development and chromosomal defects in developed oocytes.

A study on a species of killifish has shown that concentrations of 20 ppm have substantial teratogenic effect. The embryos developed several cardiovascular anomalies, and occasional eye and splenic defects. The 20 ppm treatment reduced hatchability to less than 50%, and 25 ppm allowed less than 5% to hatch. No anomalies were found after treatment with 14 ppm 2,4,5-T, and the hatch was reduced only 4% (Schreiweis and Murray, 1976).

Carcinogenic and Mutagenic Potential of 2,4,5-T

Probably the most important issue about any chemical introduced into the environment by human activity is the possibility that it may increase the incidence of cancer in the human population. The probability of muta-

genic activity is of almost equivalent concern, both because of the possibility of genetic alteration and because mutagenesis may be a useful predictor of carcinogenic activity. The relationship is by no means constant. However, McCann et al. (1975) have assembled data from a number of laboratories using microbial systems ("Ames test") and find that 85% of known carcinogens are positive, 10% of non-carcinogens are active. This is not to say that almost every agent identified as a mutagen will be carcinogenic, but the test should become an aid in deciding priorities for direct screening.

Only a limited number of experimental studies of 2,4,5-T carcinogenicity have been made. A screening program for 120 pesticides and industrial chemicals was reported by Innes et al. (1969). Eleven of the agents caused an elevated incidence of tumors; none of the phenoxy herbicides, including 2,4,5-T, caused increased tumor formation at a dose previously determined to be the maximum tolerable daily dose over 20 days, without lethality (21.5 mg/kg/day for 2,4,5-T). Muranyi-Kovacs et al. (1976) treated two strains of mice with 80 ppm 2,4,5-T (TCDD content 0.05 ppm) in the diet for more than 500 days. Daily dosage was about 12-15 mg/kg. In one strain survival time was significantly decreased in males, in the other significantly increased in females. An apparently significant small increase in tumor incidence occurred with the increase in life span. The authors are concerned that altered life span may confound the analysis, but consider that 2,4,5-T can not yet confidently be considered non-carcinogenic, and requires further analysis.

A two year study of rats given up to 30 mg 2,4,5-T/kg/day has just been terminated by Dow Chemical Company, but the pathological evaluation had not been completed at the time of this preparation.

One epidemiological study has been made of tumor incidence in a group of Swedish railroad employees. It was possible to isolate groups who had handled phenoxy acids exclusively, and a cohort of 207 persons with at least 45 days of exposure, totaling 1747 person-years. No increase in tumor incidence could be detected in these workers although increased incidence appeared in groups exposed to other herbicides (Axelson and Sundell, 1974).

Mutagenic assessments have been carried out on a number of systems. Buselmaier et al. (1973) were not able to show 2,4,5-T induced mutations in Salmonella typhimurium G46 his-, and Serratio marcescens a21 Leu- and a31 his- in a host mediated assay in mice. Serum from animals treated orally with 2,4,5-T also did not induce mutants in S. typhimurium his- (Styles, 1973). Anderson et al. (1972) were unable to demonstrate any mutagenic properties of 2,4,5-T or other phenoxy herbicides when tested against eight histidine requiring mutants of S. typhimirium.

Sex linked lethal tests of adult male Drosophila showed 2,4,5-T to be negative, although fertility was decreased (Vogel and Chandler, 1974). Majumdar and Golia (1974) were able to show an increase in sex linked recessive lethal mutations after 15 day feeding on 1000 ppm 2,4,5-T. A non-significant increase was apparent after feeding 250 ppm.

In gerbils six-day feeding of up to 150 mg 2,4,5-T/kg produced no increase in chlorosomal abnormalities, but higher doses did cause some increase (Majumdar and Hall, 1973). Jenssen and Renberg (1976) tested 2,4,5-T for mutagenicity by seeking micronuclei in erythrocytes of mouse bone marrow, which is a high resolution detection system. The system was cytogenetically negative, although some mitotic depression was detected. It was noted that only about 5% of circulating 2,4,5-T found its

way into the cells, which was suggested as indicating a limited hazard due to lack of access.

A limited survey of pesticide applicators who had been in contact with a variety of agents showed some increase in chromatid gaps and breaks during the spray season (Yoder et al., 1975). Recovery in the off season reduced frequency below that of controls, suggesting an augmented repair system. 2,4,5-T was the least frequently used agent, and it is not likely that any observed effect can be attributed to it.

Dow Chemical Company has maintained a program of continuing health monitoring of 2,4,5-T production workers. Cytogenetic evaluations of 52 men in early 1970 were reported to be negative (Johnson, 1971), and a second analysis in 1974 also found no evidence of abnormality (Kilian, 1975). An interesting finding, however, was that the fraction of each exposed group found to have no abnormal cells was above 80%, while of the control group only 74% had no abnormal cells.

Absorption, Distribution, Metabolism, and Excretion of 2,4,5-T

2,4,5-T has a rather short residence time in all species. A cow given 450 mg of 2,4,5-T acid in four daily doses excreted the entire amount in urine in six days (St. John et al., 1964). Erne (1966a, 1966b) administered 100 mg of the amine salt/kg to rats and swine and found plasma half time for the rat to be three hours, and 10 hours for pigs. Kidney, liver, lungs, and spleen concentrations of 2,4,5-T could occasionally be forced above plasma levels, but apparently none of the material entered the brain or adipose tissue. Tissue half times ranged from 5-30 hours. When the agent was administered repeatedly plasma levels tended to lessen, with increased excretion. Of the 2,4,5-T in blood, about

20% was in erythrocytes. 2,4,5-T is excreted more slowly by mice than by rats, at a rate of 1-4% of the original dose per hour (Zielinski and Fishbein, 1967).

By overloading sheep (four 250 mg doses) it is possible to produce residues in tissues. Maximum accumulations were about 100 ppm in fat and muscle. In each case residues were found in the acid form regardless of the form in which it was fed (Clark et al., 1970). The latter finding is at some variance with Clark et al. (1971), in which the PGBE ester of 2,4,5-T was reported to remain as the ester in urine and tissues. Cattle fed 0.15 and 0.75 mg 2,4,5-T propylene glycol or butyl ether esters/kg/day for 32 weeks, produced no increase over background residue. Clark et al. (1971) later reported feeding sheep 2000 ppm 2,4,5-T in the diet and finding 1.0 ppm 2,4,5-T in muscle. No residue could be detected 7 days later. Deer browsing on land treated for reforestation were found not to contain significant tissue residues; measurable 2,4,5-T was found in stomach contents, urine, and feces up to 43 days after herbicide application (Newton and Norris, 1968). The implication, again, is that sustained field intake will not cause detectable tissue residues.

Grunow et al. (1971) found about 50-70% of administered 2,4,5-T in urine, and found that a substantial fraction emerged as derivatives, one of which was identified as N(2,4,5-trichlorophenoxy acetyl) glycine. The taurine conjugate and 2,4,5-trichlorophenol have also been identified (Grunow and Böhme, 1974). Nony et al. (1976) have measured 2,4,5-T, its glycine amide and alkaline hydrolyzable conjugates in blood, urine, and feces. The proportion of metabolites is quite low in blood, and increases substantially in urine from 24-72 hours after administration of the herbicide. In the feces the glycine amide is found in only limited amounts, but other conjugates may account for as much as 25% of the total recovery.

Research with labeled 2,4,5-T in rats showed that the half-time (T/2) of plasma clearance was about 4.5 hours and essentially dose independent below doses of 50 mg/kg but increased as dosage increased to 19.4 hrs at 100 mg/kg and 25.2 hr at 200 mg/kg. The T/2 of a 5 mg/kg dose in dogs was much longer, 77 hours. About 90% of the 2,4,5-T in blood was reversibly bound to plasma protein, over a wide range of concentrations. It was found also that as the dose was elevated, a small amount of fecal excretion took place. Detectable, minute amounts of ^{14}C emerged in the respiratory gases (Piper et al., 1973). Fang et al. (1973) also showed a non-dose dependent T/2 at lower doses. Nony et al. (1976) found in excess of 10% of the total excreted material in feces. The extent of protein binding has been further defined by Kolberg et al. (1973); bovine serum albumin binds 13-14 moles 2,4,5-T/mol BSA.

As suggested by the slower T/2, the percentage of dose excreted/day decreased sharply at the higher doses, indicating a limit in excretory capacity by the kidney. Rat kidney tissue slices can concentrate 2,4,5-T about 15 times, slices from dogs can concentrate about 9 times. Berndt and Koschier (1973) found an energy dependent uptake of phenoxy herbicides by renal cortical tissue. Metabolic inhibitors depress the process. Acetate and lactate as substrates enhance the accumulation. Increased 2,4,5-T in the medium slows the concentrating capacity of the kidney.

The above work was later carried forward by resorting to intravenous injection of 2,4,5-T in order to take advantage of useful pharmacokinetic models (Sauerhoff et al., 1976). Doses of 5 mg/kg and 100 mg/kg were used, and samples were taken through 36 hours (5 mg) and 72 hours (100 mg). After 5 mg/kg rats excreted about 50% of the body burden every 12 hours, in spite of a 4.3 hour plasma T/2, further illustrating the concentrating

capacity of the kidney. At 100 mg/kg the plasma T/2 was 23.1 hours for the first 36 hours, then was comparable to the animals given a lower dose.

In humans (Gehring et al. (1973) found that a 5 mg/kg dose was excreted with a T/2 of 23 hours for both plasma and whole body clearance. Essentially all of the administered material emerged in urine unchanged.

The phenoxy acids are relatively strong organic acids, which is one of the reasons they are excreted in large part as the parent molecule. The possibility of hydrolysis by rumen flora or by tissue enzymatic processes was first considered by Wright et al. (1970) who found that after feeding of 2(2,4,5-trichlorophenoxy) ethyl-2,2-dichloropropionate to sheep, 2,4,5-trichlorophenol residues could be detected in tissues. Clark et al. (1975) measured phenoxy acid and phenol residues in muscle, fat, liver, and kidney of sheep fed 2000 ppm 2,4,5-T for 28 days, and found 1.00, 0.27, 2.29, and 27.2 ppm 2,4,5-T and 0.13, <0.05, 6.1, and 0.9 ppm 2,4,5-trichlorophenol, respectively. Seven days after withdrawal, 2,4,5-T residues were well below 0.1 ppm or below the detection limit of 0.05 ppm. The phenol remained at significant levels in both liver (4.4 ppm) and kidney (0.81 ppm).

According to Koschier and Berndt (1976c) the rate of excretion rises almost immediately to about 80% of input in animals treated daily, then continues to rise for 7-9 days until excretion approximately equals input, where it remains through the duration of treatment. The rate of administration was 20 mg/kg/day. As dose rate increased the initial percentage excreted was less, but 7-9 days was still required to reach a rate equal to input. An apparent over-shoot then took place for 5-6 days.

The markedly different rates of 2,4,5-T excretion by rats and dogs was studied in vitro by Hook et al. (1974). They found that kidney of

both species actively accumulated 2,4,5-T by a saturable, oxygen dependent process which was depressed by other organic anions. Rat kidney had a greater dependence on potassium, and dog kidney increased accumulation of 2,4,5-T in the presence of acetate. The capacity of kidney to transport the standard test anion, para-amino hippuric acid (PAH), can be competitively inhibited by 2,4,5-T, indicating that both are transported by the same mechanism. The authors concluded that the greater capacity for PAH transport by rat tissues accounts for the difference in retention between rats and dogs. Apparently lower renal excretion is also responsible for the extended half-time of 2,4,5-T in new-born rats observed by Fang et al. (1973); Hook et al. (1974) have found that kidneys of 10 day old rats are much less efficient than adults. Subsequent work by the same group (Hook et al., 1976) indicated that plasma binding in dogs was more tenacious than in rats.

The high specificity of 2,4,5-T in causing cleft palate has been investigated by Dencker (1976) as a distribution phenomenon. The sensitive period has been shown to be late in fetal development, during days 12-13 (Neubert and Dillman, 1972). Palatal closure occurs very late in organogenesis and is complete at about day 15.

Dencker (1976) points out that no unusual uptake of 2,4,5-T in these structures occurs, but overall uptake in the fetus is greatly increased between days 11 and 18. It may well be that this increase, at a time when formation of most other structures have progressed far enough that they are not sensitive, is responsible for cleft palate.

It is possible for 2,4,5-T to reach milk if a sufficient level is included in the diet. At 30 ppm in the diet for two weeks, followed by one week on untreated feed, no 2,4,5-T or trichlorophenol was found at

a detection level of 0.05 ppm; at 100 ppm trichlorophenol was barely detectable. At 1000 ppm 0.31-0.54 ppm 2,4,5-T and 0.16-0.27 ppm trichlorophenol was found in milk, and somewhat less was found in cream (Bjerke et al., 1972). These figures can be put in the perspective of forage consumption. Residues on grass are on the order of 100-150 ppm/lb/acre (Morton et al. 1967).immediately after application. These amounts drop sharply to about 20% of initial levels during the initial two weeks post-application. This factor, with the rapid excretion characteristic of 2,4,5-T make even limited human exposure through dairy products or meat highly unlikely unless products are obtained immediately after heavy spraying.

Behavior of 2,4,5-T in the Environment and Effects on Submammalian Species

One factor in the hazard potential of an herbicide is determined by its persistence after application and its movement and reactions in soil, water, or in plants.

Appearance of 2,4,5-T in forest streams has been shown to result only if the herbicide was introduced directly to the stream, and diminished from about 0.1 ppm to less than 0.01 ppm within one day after treatment (Norris, 1967). Norris (1968) also found that heavy rains six months after treatment did not move detectable 2,4,5-T into streams.

The herbicide is usually degraded on the forest floor quite rapidly. Norris (1969) found that nearly 90% disappeared in two months, although cold weather, sterile soil, and lack of moisture may extend the period of degradation over as much as 9 months. The trichlorophenol hydrolysis product of 2,4,5-T degrades faster than the parent compound (Alexander and Aleem, 1961). Evaluation of several soils from South Vietnam indicates that concentrations on the order of those expected after a typical forest application should be virtually gone in seven weeks (Byast and Hance, 1975).

More than 20% of the radioactive carbon of labeled 2,4,5-T had become incorporated in the soil, but actual residues of herbicide were usually about 1%. 2,4,5-T has also been found short lived in various Texas soil by Bovey and Baur (1972). Altom and Stritzke (1973) studied 2,4,5-T in three Oklahoma soils and found half-times of 24, 14, and 21 days. The shorter time was on grass lands, the others were forests.

Leaching tests show that 2,4,5-T bound in soil remained in the upper 6 inches of test columns even after application of 4.5 inches of water (Wiese and Davis, 1964).

The various esters of 2,4,5-T are often intrinsically more toxic than the parent acid, probably because they are more fat soluble. In an aqueous system, however, they hydrolyze rapidly to the acid, and the rate is enhanced by soil and microorganisms (Teasley and Williams, 1970, as quoted by Kenaga, 1974). It seems reasonable to expect a fairly rapid hydrolysis in any system with some water present.

In essence, 2,4,5-T disappears relatively rapidly after application, probably within two months in a forest environment. The evidence also seems firm that 2,4,5-T deposited on the ground will not move into a water course, and will not leach into deeper layers.

It is difficult to separate physical behavior after application from effects on lower organisms, because there is a very close interface between an agent bound to soil or in water, and insects or fish. Lower organisms are of direct importance in assessing environmental impact of any introduced chemical. Food chain effects may predict repercussions at higher levels, and specifically sensitive organisms may be useful indicators of pollution. Ecological displacement of any kind must be accepted only after careful examination, whether secondary to the intended

impact of the chemical on plant species or as a result of direct toxic effect of the applied chemical.

The various derivatives of 2,4,5-T differ in toxicity to fish. The parent herbicide, the amine salts and isooctyl esters are relatively limited in toxicity, but the propylene glycol butyl ether and butoxy ethanol esters are quite toxic to some species. There is a large number of gross effect studies on various aquatic species; these generally use an end point of death or immobilization. Shellfish studies measure shell growth as an index of effect.

Standardization of methods has not received as much attention as seems justified. Study periods vary from 24-96 hours, water temperatures sometimes appear outside the normal acceptable range for the species, and most evaluations seem to be carried on under static conditions. Nonetheless, the relative gross effects can be useful in reference to a given application in the field. As a very rough approximation, a 2 kg/hectare application over a water course would produce a concentration of about 2 mg/L or 2 ppm, if an assumption of dilution in the upper 10 cm of water is accepted. Flowing water will diminish the concentration below detectable limits in a few hundred yards (Evans and Duseja, 1973), and application over deeper water will dilute accordingly.

Concentrations of 2,4,5-T or its derivatives found to have no effect are listed below as acid equivalents:

	Species	Exposure Period	No Effect Acid Equivalent Concentration ppm	Reference
2,4,5-T	killifish	48 h	50	Butler, 1963
	mullet	48 h	50	Butler, 1963
	sea lamprey	72 h	2	Applegate et al., 1957
2,4,5-T Na salt	bluegill	12 d	46	Hiltibran, 1967

2,4,5-T butyl ester	trout	24 h	4.1	Applegate et al., 1957
	bluegill	24 hr	4.1	Applegate et al., 1957
	lamprey	24 h	4.1	Applegate et al., 1957
2,4,5-T isooctyl ester	bluegill	8 d	7	Hiltibran, 1967
	green sunfish	8 d	7	Hiltibran, 1967
	bluegill	12 d	0.7	Hiltibran, 1967

Median lethal concentrations of 2,4,5-T derivatives are similarly tabulated:

	Species	Exposure Period (hours)	LC_{50} Acid Equivalent Concentration ppm	Reference
2,4,5-T DMA salt	bluegill	48	144	Hughes and Davis (1963)
2,4,5-T TEA salt	bluegill	24	54	Davis and Hughes (1963)
2,4,5-T Oleic-1,3-propylene diamine salt	bluegill	48	2.9	Davis and Hughes (1963)
2,4,5-T isopropyl ester	bluegill	48	1.7	Davis and Hughes (1963)
2,4,5-T isooctyl ester	bluegill	48	31	Hughes and Davis (1963)
2,4,5-T butoxyethanol ester	bluegill	48	1.4	Hughes and Davis 1963
2,4,5-T PGBE esters	bluegill	48	17	Hughes and Davis, 1963
	spot	24	0.21	Cope, 1965
	harlequin	48	1.0	Alabaster, 1969

While the more complex esters of 2,4,5-T tend to greater toxicity in water at pH 6.5, they hydrolyze to the parent acid in about a day (Teasley and Williams, 1970, as quoted by Kenaga (1974).

Effects of 2,4,5-T on other aquatic organisms have also been measured. In flowing salt water 1 ppm 2,4,5-T caused no mortality in brown shrimp and 2 ppm was without effect on oyster shell growth (Butler, 1963). The PGBE ester of 2,4,5-T at 0.14 ppm (0.09 ppm acid equivalent) caused a 50% decrease in oyster shell growth after 96 hour exposure (Butler, 1963).

Studies of terrestrial non-mammals seem to have been limited to honey bees. Honey bees are an important component of the crop cycle and in many areas are systematically managed for pollination. Phenoxy herbicides, including 2,4,5-T, fed in 60% sucrose-water solution at 10 ppm did not affect hatching, but reduced brood development when fed at 100 ppm (Morton and Moffett, 1972). When sprayed in a water carrier at field application rates, 2,4,5-T was non-toxic. (Petroleum solvents alone caused high mortality during the first day after spraying.)

Aerial spraying did not result in herbicide accumulation in honey sacs or colonies (Moffett and Morton, 1972). The point was made that the primary damage would be failure of flowers and loss of nectar, rather than direct toxicity. When fed directly to newly emerged bees, the phenoxy herbicides were essentially non-toxic at concentrations up to 1000 ppm in the diet (Morton et al., 1972).

2,4-D

Toxicity to Humans

For some reason there have been reported several incidents of 2,4-D poisoning in humans, but virtually no reports of human intoxication by 2,4-T. Probably the best known was a suicide by a single dose of 2,4-D DMA salt of which more than 90 mg/kg was estimated to have been retained by the victim, after extensive vomiting (Nielsen et al., 1965). An

accidental poisoning with a formulation containing 49% S-ethyldipropyl-thiocarbamate, 9% kerosene, and 36.5% 2,4-D isooctyl ester caused symptoms and laboratory findings attributable to 2,4-D intoxication. Among these were twitching and paralysis of intercostal muscles, hemoglobinuria, and myoglobinuria. No evidence of peripheral neurological damage was evident during 36 months of follow-up observations (Berwick, 1970). Goldstein et al. (1959) reported on three cases in which 2,4-D esters were absorbed through the skin, resulting in peripheral neurological changes. One patient spilled 60 ml of a 10% solution of 2,4-D ester on the forearms and did not wash. Unusual fatigue developed in a few hours, and over 10 days after exposure there was extended nausea and vomiting, with considerable weight loss. A second exposure caused similar symptoms, then pain and numbness in all digits, loss of skin from the palms, and within six weeks there was substantial general neural damage. Another patient eventually developed metacarpal pain and swelling in both hands, and later became partially paralyzed. The third experienced gastrointestinal disturbance and vertigo, then paresthesia in the arms and legs and persistent general muscle fasciculations. In two of the cases, there were second exposures which appeared to cause greater impact than the initial event. Similar cases have been described by Todd (1962), Berkeley and Magee (1963), and Wilson (1956).

Seabury (1963) attempted use of 2,4-D in treatment of a terminal case of disseminated coccidoidomycosis, on the rationale that the 2,4-D as a synthetic plant hormone might alter the course of the fungus infection. At the time of the treatment (1949) there was no therapeutic agent for the disease. In 24 treatments over a period of more than a month the dosage was raised to a final treatment of 3600 mg. No prior doses, up to 2000 mg caused any response, but the final administration caused

extreme quiescence, and fibrillation of muscles in the face and hands, followed by deep stupor and reflex failure. The patient recovered from the 2,4-D within 48 hours and died of the fungus disease about two weeks later.

General Toxicity to Laboratory Animals

One of the earliest published studies of 2,4-D toxicity was by Bucher in 1948. She found an acute LD_{50} of 280 mg/kg in mice, and found that single doses of 150-200 mg/kg would produce a myotonia persisting several hours. The animals remained awake and alert, but when moved exhibited gross incoordination. They were capable of working out of the syndrome with continued exercise, but if allowed to remain quiet the myotonia would recur. The chemical also caused diarrhea in mice at the higher doses, and gastrointestinal and upper respiratory irritation in dogs. Mice were able to tolerate daily doses of 1/2 LD_{50} for three months. Hill and Carlisle (1947) established LD_{50} values for several species: mice, 375 mg/kg; rats, 666 mg/kg; rabbits 800 mg/kg; and guinea pigs, 1000 mg/kg. They also treated monkeys and were able to give up to 214 mg/kg without severe residual effect. Twice that dose caused vomiting, incoordination, and loss of muscle tone. Rowe and Hymas (1954) summarized lethality data on 2,4-D and five salts or esters; in general the lethal doses were very high, with the exception of a few dogs given 2,4-D itself, for which the LD_{50} was estimated at 100 mg/kg. The latter data was apparently derived from the observations of Drill and Hiratzka (1953). Hansen et al. (1971) conducted a two-year feeding study on rats, at dosages of 5 to 1250 ppm 2,4-D in the diet, without appreciable change in growth, hematologic values, or organ weight. In the same study dogs were maintained for two years with little effect at doses up to 500 ppm 2,4-D.

Drill and Hiratzka (1953) found that 20 mg/kg daily was fatal in 3 of 4 dogs within 49 days of a 90-day study.

Grazing species of animals might be expected to suffer exposures in the field from eating treated vegetation. Palmer and Radeleff (1964) evaluated a series of herbicides at high doses in limited numbers of ruminants. One sheep tolerated 481 daily doses of 100 mg 2,4-D alkanolamine salt, another was unaffected by the same treatment with 2,4-D propylene glycol butyl ethyl ester. The first compound was lethal after 7-500 mg/kg doses; the second was lethal after 9-250 mg doses. A bovine tolerated 112 doses of 50 mg of the alkanolamine salt, but another developed digestive problems after 80 doses of 100 mg/kg. Palmer (1963) administered daily doses of 50-250 mg 2,4-D/kg as the alkanolamine salt to a group of steers. The steer given 100 mg/kg developed ruminal atony after 86 days, presumably as a result of the herbicide. Animals given 200 and 250 mg/kg became intoxicated in 34 and 15 days, respectively, with dessicated mucous membranes and tendency to nosebleed when restrained.

Ruminants on sprayed pasture or cows given 5.5 gm daily did not show clinical evidence of toxicity, nor did production decline. 2,4-D appeared in serum of a cow fed 5.5 gm/day for 106 days, but none was passed in milk (Mitchell et al., 1946).

Chickens and other fowl also appear relatively insensitive. Bjorn and Northen (1948) found no impairment in weight gain at doses up to 28 mg 2,4-D alkanolamine/kg three times weekly for four weeks; at 280 mg/kg there was a marked decrease in gain. Single doses of 765 mg/kg were lethal, 380 mg/kg was not. 2,4-D did not cause decrease in growth rate at dose rates up to 1000 ppm in the diet, but at 7500 ppm growth essentially stopped (Whitehead and Pettigew, 1972). The acute LD_{50} was

estimated at 900 mg/kg. Whitehead (1973) tested dietary 2,4-D at concentrations up to 100 mg/kg diet, and found that at dietary levels of 10 mg/kg diet or greater, growth rate was depressed. Because food conversion efficiency was not affected, it was suggested that palatability was affected resulting in decreased consumption. Chickens will discriminate against 2,4-D contaminated food (Whitehead and Pettigrew, 1972). The U.S. Fish and Wildlife service tested a number of chemicals by feeding to various game bird species in the early 1960s. 2,4-D acetamide given to young quail at 2500 ppm caused 72% mortality in 12 days, the butoxyethanol ester at 5000 ppm caused 28% mortality in 135 days, and 2500 ppm dimethylamine salt caused 12% death in 138 days. In older birds 2500 ppm was almost without effect after 50 days, and after 111 days of 1000 ppm feeding. Tests on Coturnix indicated a similar tolerance (Stickel, 1964). More recently, Hill et al. (1975) have shown the LC_{50} for 2,4-D acetamide in the diet of bob white, coturnix, pheasant, and mallard to be in excess of 5000 ppm. The butoxy ethanol ester and DMA salt have a similar low toxicity.

A number of other biological effects of 2,4-D have been found experimentally, usually at very high doses. Dybing and Kolberg (1967) suggested that 2,4-D was actively reabsorbed, and if so would conpetitively decrease the clearance of p-aminohippurate without interfering with creatinine clearance. Studies with rabbits given 100 mg priming doses, then 2 mg 2,4-D/min produced decreased PAH clearance at doses of 62-115 mg/kg. Chang et al. (1974) studied a number of effects on rat liver following treatment with 2-5 gm/kg 2,4-D over 4-7 weeks. Glycogen content was 50-100% higher, and liver nuclei synthesized RNA more actively in vitro than

did controls. DNA content per liver was decreased. In this work, 2,4-D was compared with 2,4,5-T; the latter compound caused increased liver weight and protein accumulation, while 2,4-D decreased liver weight and lessened RNA content, with no protein accumulation. Speculation is justified that TCDD in 2,4,5-T was responsible for the difference. A dietary supplement of 60 ppm 2,4-D to lambs had no effect on rumen liquor or sheep or serum proteins. Weight gain was slightly decreased over a 12 week feeding period (Abou-Akkada et al., 1975). The same authors (1973) had previously found that 2,4-D did not alter ruminal microbial function. At fairly high doses (80 mg/kg/day, for seven days) 2,4-D increases ^{131}I intake by the thyroid. The effect only occurs in a normally functioning thyroid gland; it was not seen after hypophysectomy or iodine depletion (Florsheim and Velcoff, 1962). The effect is apparently due to lowered thyroxine binding to serum protein (Florsheim et al., 1963) but whether this is secondary to 2,4-D binding or is a specific pharmacologic effect is not known.

There has been limited in vitro study of 2,4-D. Weiss and Beckert found stimulated mitotic activity and increased chromatin after cultured monkey kidney cells, Girardi heart cells, and trout gonad cells were exposed to 10 or 50 ppm 2,4-D for 72 hours. 2,4-D inhibits mevalonate incorporation into non-saponifiable lipids of liver, but only at concentrations in excess of 1 mM (Olson et al., 1974). Oxidative phosphorylation in rat mitochondria appears to be sensitive to concentrations as low as 10^{-4} M; at 10^{-3} M respiration was normal but P/O decreased to 20% of normal (Brody, 1952). In L929 cells in monolayer culture, 2,4-D causes triglyceride accumulation when present at a concentration of 500 µg/ml (Kolberg et al., 1972) and inhibits all growth at 50 µg/ml (Kolberg et al., 1971).

2,4-D at high doses is used also as a chemical inducer of experimental myotonia, as a model of the congenital disease (Iyer et al., 1977; Eyzaguirre et al., 1948). The action is apparently due to an increase in membrane resistance and reduced chloride transport. Electrical activity of the brain was found to be reversibly inhibited by doses of 200 mg 2,4-D/kg, in rats (Desi et al., 1962). The chemical also produces a primary myopathy that is useful in study of the family of diseases which includes white muscle disease in lambs (Heene, 1969). These effects are not of toxicologic significance except in cases of massive acute intake resulting in myoneural symptoms.

Effect of 2,4-D on Reproductive Function

As with 2,4,5-T, the possibility that 2,4-D has teratogenic potential was first studied by the Bionetics Research Laboratories study (Bionetics Research Laboratories, Inc., 1970). The data were suggestive that incidence of failed lower jaw formation was somewhat greater than that resulting from the DMSO carrier. Schwetz et al. (1971) measured teratogenic and fetotoxic effects of 2,4-D and two esters on rats. The higher daily doses (75 mg 2,4-D/kg; 75 mg propylene glycol butyl ester of 2,4-D/kg; 87.5 mg isooctyl ester of 2,4-D/kg, each just below the maternal toxic dose) caused decreased fetal weight, subcutaneous edema, delayed bone ossification and wavy ribs. Most of these changes are fetotoxic rather than teratogenic. The last two are developmental effects but have no effect on survivability. No teratogenic responses were found at any dose.

There is some difference in definition of teratogenic response among authors in the field. A variety of skeletal defects that do not interfere

with postnatal survival were found by Khera and McKinley (1972); most effects observed were wavy ribs or fused sternum. The increased incidence of these changes was evident at doses as low as 25 mg/kg/day. 2,4-D teratogenesis was studied by Bage et al. (1973) but only in presence of 2,4,5-T. The mixture caused some teratogenesis, but was less effective than 2,4,5-T alone, so the impact of 2,4-D in the system was difficult to evaluate.

Hamsters are subject to a teratogenic effect of high doses of 2,4-D. Collins and Williams (1971) found that 2,4-D from three different sources caused a low incidence of anomalies, usually fused ribs, at doses of 100 mg/kg/day through days 6-10 of gestation. There was no satisfactory dose response relationship. Dietary 2,4-D at 500 and 1000 ppm did not alter reproductive function in a three-generation, 6 litter study with rats. Percentage of pups surviving to weaning and weanling weight was decreased at 1500 ppm, however (Hansen et al., 1971).

Sheep are apparently not subject to 2,4-D induced teratogenesis. Binns and Johnson (1970) administered 2 grams daily for 30, 60, and 90 days following breeding and caused no malformation. There were presumably 6 sheep per group but the number in this specific experiment was not stated.

Eggs are peculiarly vulnerable to exposure to herbicides and several studies have been directed toward defining the hazard of 2,4-D to chicken or game bird eggs. An injected dose of 10 mg 2,4-D/egg caused 50% mortality, 5 mg/egg resulted in 30% loss, and at 0.5 mg/egg 90% of the eggs hatched. No deformities occurred at any dose (Dunachie and Fletcher, 1967). A later study by Dunachie and Fletcher (1970) confirmed these findings for 2,4-D and 2,4-DB (2,4-dichlorophenoxybutyric acid).

In contrast, Lutz-Ostertag and Lutz (1970) sprayed pheasant and grouse eggs with 2,4-D at rates commonly used in the field and caused embryonic mortality and terata. Somers et al. (1973, 1974) were not able to support these findings after spraying mixtures of 2,4-D and picloram at usual field rates (2.8 kg/ha) and 2,4-D and 2,4,5-T on hen eggs (1973) and pheasant eggs (1974) at 10 times field concentrations (11.2 kg/ha). They found no "adverse effect on hatching success, incidence of malformed embryo or subsequent chick mortality". Kopischke (1972) also found no effect on pheasant eggs sprayed at field concentrations, but found that diesel fuel as a carrier blocked hatching completely. Dipping hen eggs in 1% 2,4-D for 10 seconds, then continuing incubation was similarly ineffective (Gyrd-Hansen and Dalgaard-Mikkelsen, 1974).

An interesting observation of 2,4-D distribution in mouse fetuses has been made by Lindquist and Ullberg (1971). Labeled 2,4-D given late in gestation accumulated early in the yolk sac, passed on to the fetus and was almost completely eliminated by 24 hours after administration. Distribution among tissues was non-selective, and concentrations tended to parallel those of the dam, perhaps explaining in part the lack of teratogenic effect.

Carcinogenic and Mutagenic Potential of 2,4-D

Innes et al. (1969) screened 120 compounds for tumorigenic properties in mice. 2,4-D and several of its esters were included; none caused increased tumor incidence. Hansen et al. (1971) carried out a carcinogenesis study in rats and concluded that although tumors were found, the observed incidence did not support a finding that 2,4-D is carcinogenic. There seemed to be some inconsistencies in interpretation that may have confused the issue. Apparently no other evaluations of cancer

potential of 2,4-D have been made. A number of mutagenic screens have included 2,4-D, however. Jenssen and Renberg (1976) found that 2,4-D would not induce increased micronuclei in mouse bone marrow erythrocyte, but the compound did slightly depress mitotic activity. Sex-linked lethality assay of 2,4-D in male Drosophila was also negative for mutagenic activity (Vogel and Chandler, 1974). Styles (1973) treated rats with 2,4-D, then used serum from the animals in a host mediated assay with histidine-requiring S. Typhimurium mutants. No effect of 2,4-D was evident. In a screen of 110 compounds with the "Ames test," using eight histidine-requiring mutant strains, Anderson et al. (1972) was unable to detect mutagenic activity by 2,4-D.

Absorption, Metabolism, Tissue Distribution, and Excretion of 2,4-D

2,4-D was absorbed rapidly from the lung of rats in which the herbicide was injected intratracheally as 0.1 ml of 0.01-10 mM solution. The animals were sacrificed from one half to 120 minutes later. The half-time for 2,4-D absorption was 1.4 minutes, and concentration had little influence on the rate (Burton et al., 1974). When ingested, 2,4-D is absorbed primarily by the portal circulation; the lymphatic drainage accounts for very little 2,4-D absorption, as might be expected from the limited fat solubility of the herbicide (Sieber, 1976). Blood concentration of ^{14}C from radiolabeled 2,4-D administered orally to sheep has been shown to rise very rapidly (Clark et al., 1964). The rate of absorption of 2,4-D from the rumen is not known; the immediate elevation in blood ^{14}C suggests that at least some material was removed directly from the rumen. However, Gutenmann et al. (1963) fed 5 ppm 2,4-D to cattle and found that the concentration in the rumen contents decreased from 3.5 to 0.5 ppm over a 24 hour period, which does not suggest rapid

absorption, but rather dilution with passage of rumen content. The chemical did not disappear in an artificial rumen.

Kohli et al. (1974) gave 5 mg orally to six human volunteers and found peak plasma concentrations by seven hours; the average plasma concentration one week later was about 10% of the peak concentration. Half time for excretion was calculated at 33 hours.

Clinical observations of human patients who had extensive skin contact with 2,4-D showed systemic toxic responses within a few hours (Goldstein et al., 1959; Todd, 1962), indicating ready absorption through the skin.

Application of 2,4-D as the DMA salt, isooctyl ester and butyl ester to the body surface of rabbits was relatively ineffective (Kay et al., 1965). Treatment was with 15 ml of aqueous or oil solution applied on a 4 x 3 gauze patch, covered tightly with plastic film. Contact was seven hours daily, five days a week for three weeks; concentrations of 2,4-D were 0.626 and 3.13% acid equivalent. Some animals were treated on abraded skin. A few animals died during the experiment but the pathology and symptoms were not typical of 2,4-D poisoning; most of the fatalities were among the animals with skin damage. No neurological lesions were found, nor were any other observed parameters changed. The only lesions observed were at the site of application.

There seems to be general agreement, other than the report by Kohli et al. (1974), that significant amounts of 2,4-D do not remain in an animal for much more than one or two days. Zeilinski and Fishbein (1967) compared whole body residence times of two esters of 2,4-D and the 2,4-D acid after subcutaneous injection of 100 mg/kg to mice. Of the butyl ester, 95% was gone after 6 hours, and in animals that had been pre-

treated with five similar daily doses, the rate of disappearance was further enhanced. The isooctyl ester was considerably slower to disappear, with a half time of somewhat less than four hours, and the acid itself was retained slightly longer. The authors did not specifically note whether hydrolysis of esters to 2,4-D acid was identified as part of the metabolic process, but the method appeared to account for the disappearance of all forms of the compound. As a comparison, the disappearance half-time of 2,4,5-T acid was on the order of 20 hours.

Khanna and Fang (1966) administered 2,4-D-^{14}C to male and female rats at very high (80 mg/rat) and relatively low (1 mg/rat) doses and found tissue residues to be greatest at about 6 hours, and almost undetectable by 30 hours after treatment. Extremely high concentrations were found in the stomach but there was no information about separation of stomach content from the tissue proper. No label was found in respiratory gases. A very small fraction of urinary label was found to be an unidentified metabolite, but the data indicate that almost all 2,4-D is excreted without change by rats.

Although 2,4-D binds reversibly to bovine serum albumin (Kolberg et al., 1973) and therefore probably to other plasma proteins, there seem to be no specific tissue binding sites as are found in plants. Morre (1974) has examined fish muscle and rat liver after exposure to 2,4-D, seeking specific binding sites and has concluded that none exist.

2,4-D apparently does not move into milk of lactating animals in significant amounts, whether treated by direct administration of the herbicide or by grazing on treated land (Bjerke et al., 1972; Gutenmann et al., 1963; Bache et al., 1964; St. John et al., 1964; Klingman et al., 1966). Under forcing conditions, however, (1000 ppm 2,4-D in diet), the

level of 2,4-D residue in cows milk was forced up to 0.06 ppm, and were still barely detectable seven days later.

A substantial capacity for conjugation of 2,4,5-T has been described elsewhere in this report (Nony et al., 1976). Grunow and Böhme (1974) have found that the ability to conjugate 2,4-D with glycine and taurine exists but is considerably less than that for 2,4,5-T. In dogfish and flounder, however, a major fraction of urinary 2,4-D is excreted as the taurine conjugate (James and Bend, 1976), and in three of the four dogfish studied 10-20% of the label was found in bile after 48 hours.

In spite of the rapid loss of 2,4-D by fish, persistence of the herbicide or its products may be prolonged. Treatment of pond weeds with up to 9 kg/ha usually left no detectable residues in fish by 28 days post-application, even at the highest rates (Schultz and Harman, 1974). Schultz (1973) has found, however, that some unidentified products may be present in fish 2-3 months after treatment.

The rapid appearance of intact 2,4-D in urine is apparently mediated by a presumably active and saturable tubular excretion mechanism (Erne and Sperber, 1974). The extensive excretion of 2,4-D in urine has been noted in steers (Lisk et al., 1963), sheep (Clark et al., 1964), rats (Sieber, 1976), and humans (Kohli et al., 1974), and apparently is rapid enough to remove all but massive exposures before significant damage can occur.

Behavior of 2,4-D in the Environment and Effects on Submammalian Species

This report is primarily concerned with effects of herbicides on non-plant species, but a cursory qualitative survey of literature on field persistence is useful in judging how long a given amount of herbicide may remain available.

Water concentrations are a major concern, in relation both to aquatic species and to water supplies for the human population. 2,4-D applied in a watershed area appears in streams to a very limited extent (White et al., 1976; Krammes and Willetts, 1964). Movement into subsurface water flows also appears negligible (White et al., 1976). In aerated lake water 2,4-D persists up to 120 days, but in lake mud 2,4-D hydrolyzes very rapidly, because of microbial activity (Aly and Faust, 1964). Half-time of 2,4-D disappearance seems overall to be much less than two weeks in aqueous systems.

For some applications on water, relatively massive applications of up to 40 lbs. per acre are necessary. Two reservoirs on the Tennessee River were treated at 20 and 40 lbs/acre depending on the nature of the water milfoil infestation. The treatments did not affect fish or other fauna, and had little adverse effect on most other plants. Plankton did retain significant amounts of herbicide over extended periods, and 2,4-D was detectable on occasion in finished domestic water from the reservoirs (Wojtalik et al., 1971).

In soils, 2,4-D has been found to have a half-time of 4-5 days by Altom and Stritzke (1973). White et al. (1976) found that 95% of 2,4-D in the top 0.5 cm of soil would disappear in 7 days. However, an *in vitro* study by Alexander and Aleem (1961) indicated that 2,4-D would be detectable for as much as 94 days in one type of soil and only 23 days in another. The preparation included 100 ml of nutrient medium and 4 mls of soil as an inoculum; whether such a system represents actual breakdown conditions in soil is possibly questionable. Sufficient material to cause detectable phytotoxicity persists for about a month, according to Mullison (1972). In areas where 2,4-D is used annually, the breakdown may be more rapid in the later years than following the first

treatment (Hurle and Rademacher, 1970). Wiersma et al. (1972) reported a 1969 national program for soil monitoring in which three samples from a total of 28 which had been treated with 2,4-D were found to have from 0.01 to 0.03 ppm 2,4-D present. It seems unlikely that 2,4-D would persist in either water or soil for more than a month.

2,4-D has been used extensively in control of aquatic weeds, particularly water hyacinth and water milfoil, and has probably been subjected to more study of aquatic toxicity than any other herbicide. A number of short term screens have been conducted with fish. Alabaster (1969) reported on 164 compounds, including 2,4-D sodium salt, which was found to have a 24 hour LC_{50} of 1160 ppm for harlequin fish, and the butoxyethylester of 2,4-D which was much more toxic with a 24 hour LD_{50} of 1 ppm, for the same species.

The 24-96 hr LC_{50} of PGBE esters of 2,4-D for rainbow trout were slightly above 1 ppm (Cope, 1964). For mullet and killifish the LC_{50} (24 hr) was 5 ppm for both the PGBE and butoxyethanol esters. The parent acid was ineffective at 50 ppm (Butler, 1963).

Oyster shell growth was 50% inhibited by 3.75 ppm butoxyethanol ester, but the acid and dimethylamine salt were not effective at 2 ppm (Butler, 1963). Exposure to the DMA salt for 24 hrs at 2 ppm caused no mortality in shrimp; 48 hour exposure caused 10% lethality. The butoxyethanol and PGBE esters caused no mortality to shrimp at 1 ppm x 48 hour. The ethyl hexyl ester of 2,4-D caused 38% decrease in oyster shell growth at 5 ppm over 96 hrs (Butler, 1965). The considerable differences in 2,4-D effect among various species were illustrated by Sanders (1970a) 2,4-D acid has a 48 hour median response concentration (TL_{50}) of 100

mg/L (100 ppm) for Daphnia and 3.2 mg/L for scud. (Crosby and Tucker (1966) found the same value for Daphnia.) The PGBE ester TL_{50} varies from 0.1 for Daphnia to 2.6 for scud to more than 100 ppm for crayfish. The DMA salt TL_{50} is more than 100 ppm in bluegill but TL_{50} for the butyl ether ester in bluegill is 1.1 ppm, and 100 ppm for crayfish.

Stonefly naiads are less sensitive to 2,4-D itself (LC_{50} x 96 hr = 15 ppm) than the butoxy ethanol ester (LC_{50} x 96 hr = 1.6 ppm) (Sanders and Cope, 1968). Sanders (1970b) has also reported a 96 hr TL_{50} for 2,4-D amine of 100 ppm for tadpoles.

The lethal effect on tadpoles is apparently limited, but Buslovich and Borushko (1976) found that 2,4-D sodium salt would inhibit metamorphosis of Rana temporaria tadpoles, and blocked thyroidin stimulation of the process. The DMA salt was effective at 2 ppm. The authors speculate that 2,4-D antagonizes thyroid hormone.

Few studies of truly chronic exposure of fish to 2,4-D have been made. Mount and Stephan (1967) compared the 96 hour LC_{50} for 2,4-D butoxyethanol ester (5.6 ppm) with various 10 month exposures, and found that 1/19th of the LC_{50}, or 0.3 ppm could be tolerated by fathead minnows without effect on growth or reproduction. Eggs were much more sensitive than adults over 48 hour exposures. Schultz (1973) placed bluegill, channel catfish, and largemouth bass in solution of 2,4-D DMA salt, labeled with ^{14}C, at concentrations of 0.5, 1.0, or 2.0 mg/L (ppm). Fish and water samples were removed at intervals up to 84 days for analysis of ^{14}C and 2,4-D content.

Considerable radioactivity remained in fish tissues but apparently none was associated with 2,4-D, suggesting extensive metabolism. The

first tissue in which radioactivity appeared was the gall bladder of catfish and bluegills, and eventually ^{14}C appeared in every tissue analyzed. In both catfish and bluegill, the amount of radioactivity in muscle tended to increase through the collection period. No toxicity was evident in any of the exposed fish.

To relate concentrations of one ppm to field condition, an application of 2 kg/hectare (10000 M^2) to water will give a concentration of 2 mg/L or 2 ppm if the water is 10 cm deep. Deeper water will result in further dilution. If applied to flowing water, water movement will dilute the herbicide quite rapidly, and that material distributed on soil and vegetation will tend to remain in place. Obviously a spill or other accident presents different problems.

Among insects, the effects of 2,4-D on honey bees has received the most attention. Palmer-Jones (1964) has listed a number of investigators who concluded that 2,4-D is safe for bees, as well as others who have shown toxic effects, some of which may depend on the species of plant on which the herbicide is deposited. In a field investigation of a three lb/acre application Palmer-Jones found that a 22% mortality occurred in 48 hours after dusting. However, when bees at the hive were heavily dusted directly, there was no mortality, leading to the conclusion that the bees in the field were acquiring intoxicant by some mechanism other than surface contact. Moffett et al. (1972) sprayed caged bees directly with various phenoxy herbicides at a one pound/acre rate with very little effect. When included in the diet at concentrations up to 100 ppm, 2,4-D did not decrease lifetime of bees and the ester used was ineffective at 1000 ppm (Morton et al., 1972). The herbicide does cause decreased brood development when fed at 100 ppm but has no reproductive effect at 10 ppm (Morton and Moffett, 1972).

Treatment of Coccinellid beetle larvae with 2,4-D at a rate equal to 8 oz. acid equivalent/acre caused greatly increased mortality, regardless of the age of the larvae through day 12 at time of spraying (Adams, 1960).

SILVEX

There appears to be a much more limited accumulation of data on biological effects of silvex and related compounds than is available for other phenoxy herbicides. The descriptions of biological effects of silvex will be categorized in two broader sections rather than the more detailed entries prepared for the other agents.

Biological Effects of Silvex and Its Derivatives

The acute median lethal dose of silvex and its esters is quite high, and falls in a relatively narrow range among species and among the derivatives. Rowe and Hymas (1954) summarized data which had been established at that time. Silvex and its mono-, di-, and tri-propylene glycol ether esters were of almost identical LD_{50} in guinea pig of 1200 and 1350 mg/kg. The lethalities to rats were also similar at about 650 mg/kg. The rat appears to be most sensitive, and chicks and mice require doses similar to the guinea pig.

Subchronic (90 day) studies of rats fed 10-600 mg/kg/day of the PGBE ester resulted in more than 50% lethality at the highest dose. The time of death ranged from 15-85 days. Growth was depressed in rats fed 300 mg/kg. There is somewhat of a paradox in the findings, because pathology in the dead animals indicated malnutrition rather than herbicide toxicity. A paired feeding study at 300 and 600 mg/kg/day indicates that depressed growth was not entirely due to inadequate food consumption. Dose rates of

30 and 100 mg/kg/day caused an increase in liver weight but 10 mg/kg/day caused no change. (Mullison, 1966). The study was followed by a 90 day feeding of up to 10000 ppm silvex (sodium salt) in the diet. 10000 ppm is 1% of the diet, and if the animals consumed 10 gm daily and weigh 300 gm, the dose rate would be on the order of 300 mg/kg/day. That dose rate was highly lethal and was abandoned, but did produce swelling and granular degeneration of hepatocytes and ultimate cellular necrosis. Renal tubule cells were swelled and vacuolated, and seminiferous tubules were degenerated. At levels above 10 ppm (about 3 mg/kg/day) some growth depression occurred. Mullison (1966) also fed Kurosol (potassium salt of silvex, containing 53.3% silvex acid) for two years at concentrations up to 300 ppm. The highest dose caused a slightly increased kidney/body weight ratio in males, but 100 ppm and lower dose rates caused no change in food consumption, growth, gross and microscopic morphology hematology or bone marrow. 100 ppm was stated by the author to be equivalent to 2.6 mg/kg/day of silver.

Kurosol was without effect on beagles at a feeding rate of 56 ppm (about 19 mg/kg/day) over 2 years. Females fed 190 ppm suffered some hepatic degeneration and necrosis after a year of feeding, but at the end of two years no damage could be found. At the 56 ppm intake, there were no changes over a very broad spectrum of hematologic, clinical chemistry and morphologic analyses.

The liver damage at higher levels included hepatocyte necrosis, bile duct proliferation, and bile pigment deposition throughout the liver and in the epithelium of kidney tubules.

In a subchronic study in cattle by Palmer et al. (1964), one yearling Brahma-cross fed 100 mg silvex/kg/daily died after 29 days. Two other

animals given 25 and 50 mg/kg for 73 days showed no evidence of toxicity. A 90 day experiment with 50 mg/kg/day, either by drench or by injection into a rumen fistula resulted in death of one of the latter group of three animals. Two of those treated orally developed severe inflammations in the parotid area, apparently due to local irritation.

The survey by Innes et al. (1961) screened silvex against two strains of mice for 18 months. The concentration of silvex was 121 ppm, which was the maximum tolerable dose rate, and there was no increase in tumors in either strain. The mutagenic screen by Anderson et al. (1972) did not detect point mutations resulting from silvex treatment in either T_4 phage or S. typhimurium (histidine requiring).

Silvex is teratogenic at high doses. Courtney (1975) found that 398 mg/kg/day, on days 12-15 of gestation, caused 3% cleft palate in the group given silvex in DMSO subcutaneously, and 7% where given orally in corn oil. Fetal mortality increased to 25% in the group treated subcutaneously.

The Dow Chemical Co. has also conducted a series of teratology studies with silvex. Dose rates of 75, 100 and 150 mg/kg/day from day 6 to day 15 caused several cardiovascular anomalies; 50 mg/kg/day caused retarded ossification in the sternum and skull.(Dow Chemical Co., 1972). The no adverse effect level was considered to be 25 mg/kg/day. The PGBE ester caused skeletal changes at an intake of 50 mg/kg/daily but no changes were found after 35 mg/kg/day (23 mg/kg silvex acid equivalent).

Birds seem peculiarly resistant to the herbicides. For example, DeWitt et al. (1963) fed 5000 ppm BEE ester of silvex to young bob white quail, pheasant and mallard ducks. The average lethal intakes were 9350, 9240 and 21,000 mg/kg, and time of death varied from 10 to 100 days.

Mallards were able to consume 100 ppm for 100 days with no lethality, but reproduction was impaired.

Silvex acid at 5000 ppm in the diet was tolerated for 5 days with no mortality by Coturnix. The 5 day LD_{50} for pheasants was 4500 ppm of silvex BEE ester, for bobwhite quail the LD_{50} was 30 ppm over 5 days, for Coturnix it was in excess of 5000 ppm, for pheasants LD_{50} was less than 3000, and no mallards died after 5 days on 5000 ppm. All birds were two weeks old at the initiation of the study (Heath et al., 1972).

Stickel (1964) reported US Fish and Wildlife studies in which bobwhite were fed 1000 ppm of an unspecified silvex ester; 50% were dead by day 34 with average total dose of 17000 mg/kg. Of a group fed 5000 ppm, the average daily dose was 2300 mg/kg. Half of the birds survived more than 4 days, 40% survived more than 10 days, and an additional 4% died during the remaining 14 days. The average dose to birds that survived 24 days was 54,500 mg/kg.

These findings indicate that wildfowl should not be adversely affected by any field application of silvex, or for that matter even a gross over-application.

Effect of Silvex on Aquatic and Invertebrate Species

Silvex has become an herbicide of choice for control of surface and underwater water needs. As a consequence, there has perhaps been more attention paid to its impact on aquatic species than any of the other phenoxy herbicides. There is an unfortunate diversity of experimental methods and conventions for expressing concentration and other factors which sometimes makes comparison of data difficult.

Table 1 has been constructed to simplify consideration of the various reports of toxicity to aquatic species.

Table 1. Gross Toxicity of Silvex and Its Derivatives to Fish

Species	Form of Silvex	Concentration (Acid Equiv.) ppm	Effect	Period	Reference
Bluegill	BEE ester	0.36	LC_{50}	48 h	Cope, 1964
	BEE ester	1.7	LC_{50}	48 h	Hughes and Davis, 1966
	Isooctyl ester	1.4	LC_{50}	48 h	
(young)	K salt liquid	83	LC_{50}	48 h	Hughes and Davis, 1965
	K salt granular	100	LC_{50}	48 h	Hughes and Davis, 1965
(fry)	PGBE ester	0.3	tolerated	96 h	Jones, 1962
	K salt, liquid	100	tolerated	96 h	Jones, 1962
	K salt, granular	150	tolerated	96 h	Jones, 1962
(eggs)	PGBE ester	10	no effect on hatch		Wilber and Whitney, 1973
(fry)	PGBE ester	5	100% lethal	36 h	Wilber and Whitney, 1973
	PGBE ester	1	no effect	80 days	Cope, 1964
	PGBE ester	3	liver degen.	2 weeks	Cope, 1964
	K salt	10	no effect	2 1/2 mo	Cope, 1964
	K salt	75	35% lethal	2 1/2 mo	Cope, 1964
Stoneroller	K salt	25	95% hatch	72 h	Hiltibran, 1967
(eggs)	PGBE ester	5	45% hatch	72 h	Hiltibran, 1967
Chorus frog	BEE	20	LC_{50}	24 h	Sanders, 1970b
(tadpole)	BEE	10	LC_{50}	96 h	Sanders, 1970b
Fowler's toad	BEE	22	LC_{50}	24 h	Sanders, 1970b
(tadpole)					

Among factors that influence toxicity to fish are the chemical form of the agent, whether the formulation is granular or liquid, and water hardness. Generally the esters are more toxic, and liquid formulations are more toxic. Data on water hardness is contradictory; but the tendency to greater toxicity in soft water seems to be associated with the salt rather than the esters (Surber and Pickering, 1962).

Distribution rates for silvex treatment of aquatic weeds may be as high as 40 lbs/acre. An assumption of dilution throughout an 8 foot depth is apparently conventional, which would give a concentration of 1.8-1.9 ppm. If this concentration of an ester formulation were to hold in a given area, it would clearly be lethal to many fish. The salt should have up to a 100 fold safety factor for fish, depending on mineral content of the water. Such application is not a consideration in forest operation, but accidental overwater dilution could occur. Terrestrial application is at rates at least 10 fold less than 40 lb/acre but many water courses are much shallower than 8 feet, thereby leaving a similar hazard. If flowing, dilution will be rapid and periods of exposure should be limited. In static water the agent will diffuse away from the application site with time except in small shallow ponds, where some damage might possibly occur

Lower aquatic organisms have also been well studied because of silvex use on water weeds. Representative though not comprehensive data is compiled in Table 2. Again, it appears that the salts of silvex are much less toxic than the more complex esters. The most vivid contrast is in the effect on Daphnia magna, which is 50% immobilized by 100 ppm silver potassium salt, and by only 0.18 ppm silvex PGBE ester. Some species, however, are particularly resistant to even the PGBE ester of

Table 2. Gross Toxicity of Silvex and Its Derivatives to Invertebrate Aquatic Species*

Species	Form of Silvex	Concentration (Acid Equiv.) ppm	Effect	Period	Reference
Daphnia magna	PGBE ester	0.18	LC_{50}	48 h	Sanders, 1970a
	BEE ester	2.1	LC_{50}	48 h	Sanders, 1970a
	K salt	100	IC_{50}	26 h	Crosby and Tucker, 1966
Scud	PGBE ester	1.8	LC_{50}	24 h	Sanders, 1970a
	PGBE ester	0.8	LC_{50}	96 h	Sanders, 1970a
	BEE ester	1.2	LC_{50}	24 h	Sanders, 1970a
Seed Shrimp	PGBE ester	0.2	LC_{50}	48 h	Sanders, 1970a
	BEE ester	4.9	LC_{50}	48 h	Sanders, 1970a
Glass Shrimp	PGBE ester	3.2	LC_{50}	48 h	Sanders, 1970a
	BEE ester	8.0	LC_{50}	48 h	Sanders, 1970a
Sowbug	PGBE ester	0.5	LC_{50}	48 h	Sanders, 1970a
	BEE ester	40	LC_{50}	48 h	Sanders, 1970a
Crayfish adult	PGBE ester	100	LC_{50}	48 h	Sanders, 1970a
Daphnia pulex	PGBE ester	210	IC_{50}	48 h	Sanders and Cope, 1966
Simocephalus Serrulatus	PGBE ester	2.4	IC_{50}	48 h	Sanders and Cope, 1966
Stonefly naiad	acid	5.2	IC_{50}	24 h	Sanders and Cope, 1968
		.34	IC_{50}	96 h	Sanders and Cope, 1968
	PGBE ester	5.6	IC_{50}	24 h	Cope, 1965
		.34	LC_{50}	96 h	Cope, 1965
Brown shrimp adult	PGBE ester	0.28	LC_{50}	24 h	Butler, 1965

LC_{50} median lethal concentration

IC_{50} median concentration to cause immobilization

*all first in star unless otherwise noted

silvex. The crayfish LC_{50} is in excess of 100 ppm; for a number of other organisms this factor is less than 1 ppm.

It is not necessary to catalogue the entire literature on silvex effects on either fish or other aquatic species. The range of effective concentrations is well illustrated as well as the extent, if not details, of species differences.

Effects of silvex on terrestrial insect life have been evaluated in honey bees by USDA workers at Tucson. All of the phenoxy herbicides including silvex were found to be relatively non-toxic to bees when applied in water at field concentration (Morton et al., 1972; Moffett et al., 1972). When fed at 100 or 1000 ppm, silvex reduced brood production, but at 10 ppm, no adverse effect was found. In each case, when the toxicant was removed, the colonies regained their original reproductive efficiency (Morton and Moffett, 1972).

Metabolic Fate of Silvex in Mammals

There have been few studies of the disposition of silvex after absorption by mammals. Bjerke et al. (1972) fed silvex at 1000 ppm in feed and found 0.12 ppm 2,4,5-trichlorophenol (TCP) in milk and 0.16 ppm in cream. In one week off contaminated feed the concentrations decreased below 0.05 ppm. Leng (1972) in a review, reported work in which 2,4,5-TCP was not detected; however, residues of silvex were substantial in the liver. After feeding 300 ppm silvex 28 days, 4 ppm were found in liver, 18 in kidney, 0.6 in muscle and 0.9 in fat. After 2000 ppm in the diet, 12, 30, 2 and 4 ppm were found in the respective tissues. In a similar experiment Clark et al. (1975) found roughly similar values, with traces of 2,4,5-TCP in each tissue. In a single cow experiment, 5 ppm

kuron was fed for four days at 5 ppm (St. John et al., 1964). The kuron hydrolyzed to silvex and was excreted as a salt in the urine. Urinary concentrations peaked at 4.4 ppm on day 4 and declined to 0.32 on day 6. About 67% of the total fed was found in urine; feces were not analyzed. No detectable residues of Kuron or silvex were found in milk and no kuron as such entered the urine.

The pharmaco-kinetics of silvex have been studied in rats given 5 or 50 mg/silvex/kg. The compound was ring-labeled with ^{14}C. The higher dose appeared to be saturating because plasma clearance was not linear, as was the case at 5 mg/kg. Half-time for plasma clearance was 9-10 hours at both doses. At the lower dose 76% of the labeled material emerged in bile in 72 hours and 90% of the high dose. Almost all of the biliary excretion was in the first 24 hours. Most of the material was subject to entero-hepatic recirculation and eventually left the body in urine. Total silvex excreted in 192 hours in urine was 78%, and 16% appeared in feces (Sauerhoff et al., 1977a).

Sauerhoff et al. (1977b) have also examined silvex disposition by humans. One mg/kg was given orally to seven men and one woman, and plasma urine and feces were analyzed for 168 hours. Clearance from plasma and appearance in urine were bi-phasic, each stage following apparent first-order kinetics, maximum plasma concentrations occurred in 2-4 hours, 65% of the dose was excreted in urine in 24 hours. Half times for the two phases in plasma were 4 and 16.5 hours and in urine were 5 and 26 hours. The largest amount recovered in feces was 3.2%.

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In such a report as this it would be highly desirable to prepare a hazard analysis for each compartment or trophic level in the potentially affected ecosystem. This is at least very difficult and perhaps impossible to develop in a single document with the complexities of inter-level dependence and ultimate dependence on many target plant species. There is an extensive literature on such effects, but in this report a decision has been made to concentrate on potential human impact. In that process, essential information on lower species appears to the extent necessary.

There is an extensive literature on persistence, physical transport, and degradation mechanisms of the phenoxy herbicides in the environment and their behavior is well established. These areas are discussed only in sufficient detail to establish essential facts. The section on TCDD is an exception and includes as complete a treatment as possible because of the unique character of the chemical. Furthermore, the author is not competent to critically analyze the entire literature on physical and chemical behavior.

Problems Associated With Assessment of Human Health Hazard Associated With Use of Phenoxy Herbicides

There are fundamental differences of opinion in philosophy of herbicide usage. A significant segment of the population consider insertion of any synthetic chemical into the environment as fundamentally wrong, regardless of benefits, however well documented. There are as well groups and individuals who are unable to accept any suggestion that some chemicals, as used successfully and with apparent impunity for decades, might constitute a hazard which is only now becoming evident.

This issue is no more clouded by those viewpoints than is any other debate over chemical usage, and we are best served by dealing with the problem without those extremes.

Without question, the issue of phenoxy herbicides use has evolved to concerns about the contaminant TCDD in 2,4,5-T and silvex. Though 2,4-D has no such impurity, it has become tarred with the same brush, and it is not unrealistic to devote more space to the contaminant than any of the primary chemicals.

Because of the extremely low environmental levels and enormous intrinsic toxicity of TCDD, the most critical technical question about that chemical centers on analytical methodology. There are few laboratories with the sensitivity to deal with concentrations on the order of 10 ppt or less, and there is real disagreement about reliability of detection or measurement at such low levels. When the data are considered, some samples in which TCDD was undetectable had high detection limits, some areas with no spray history were found to have measurable TCDD. Also, many samples of similar origin differed widely. Nonetheless, the data appear to be telling us clearly that some TCDD is present in some segments of the environment, that the amounts are uncertain, and that existing residue information must be augmented if we are to understand the behavior of TCDD. With any effort presently imaginable, however, it will probably not be possible to directly monitor the amount of TCDD in the physical environment.

A proper analysis of hazard requires consideration of chemical behavior and ambient levels of the potential intoxicant, but in the case of TCDD we must rely on indirect assessment. The extent of uptake in

organisms that might concentrate TCDD, as compared with residues following known laboratory exposures is of some value, but is still inadequate.

There is a pervading argument over the problem of a specially sensitive or idiosyncratic individual who responds to much lower doses than the surrounding population, or who responds in a highly exaggerated manner. Such cases are so rare that usually no field association with the causative event can be made. The effect is virtually impossible to find experimentally because it may have never been identified, and if it had, the genetic and species factors probably prevent any useful modeling. The argument about idiosyncracy follows every chemical in commerce, and society has not learned how to cope with it, or even whether it wants to try. In the context of excessive sensitivity there seems to be no reason to treat the present group of chemicals differently from any others. That is to say, every reasonable attempt must be made to locate or predict adverse effect, but it does not seem possible to anticipate the extremely rare response in areas not supported by research findings.

A hazard analysis dealing with TCDD may or may not be strengthened by a position that EPA will apparently take; i.e., that 80-200 ppt TCDD in beef fat is an "effect level." The EPA document in question is as yet in draft form and will not be specifically attributed or referenced in this report. If the figures indicated are correct, any levels in beef thus far detected are within levels which will not do harm. The conclusions were reached with the assumption that beef fat will constitute no more than 2.5% of the human diet. The concern in present forestry usage is primarily with deer which browse on treated foliage, and there is no clear indication of TCDD levels which might be expected in these animals.

The most difficult problem of the several that are a part of the task of hazard assessment is, again, part of the decision pattern for any chemical, the risk-benefit analysis. Risk benefit analysis is an excellent basis for conversation but nearly useless in decision making. In the present case there seems to be a general conviction that herbicide application is a highly useful tool in forest production. At the same time, estimates of differences in actual relative costs of tree production or retail materials, either near-term or long-term, are difficult to find. Technically, economic benefit should be an accessible factor, but it seems elusive.

Risk, on the other hand, is not quantifiable unless major and obvious effects occur; such situations make the decisions for us. If we are able to show any human illness resulting from environmental practices of chemicals, almost certainly they will be disqualified. An exception may be in effects which are certainly reversible.

As a society we have yet to learn a usable method of determining whether, or how much, human injury can be considered acceptable.

The assessments of hazard in this report are therefore opinions by one person about the potential for human harm resulting from distribution of the chemicals at minimum levels that will achieve the forestry objective. This document should be taken as a working base enabling incorporation of other opinions, and it should be subject to periodic updating, based on new findings, existing papers inadvertently missed in review, and existing papers which may become more pertinent in the light of other new findings.

Assessment of Human Hazard Resulting from TCDD as a Contaminant of Phenoxy Herbicides

Two arguments emerge frequently in discussion of TCDD. One is that the manifest injuries to workers and citizens in or near sites where trichlorophenol plants have had run-away reactions or explosions dictates a cessation of use. The several such accidents, some near catastrophic are certainly unique because of the enormous toxicity of TCDD. The argument has merit but the issue here is whether specified uses are hazardous. There is little doubt that industrial safety often lags far behind existing capacity for safety, but specific industrial hygiene and health problems should be dealt with on the basis of their own weaknesses. In other words, solving the factory and community exposure problem by plant shut down may make sense, but solving it by stopping use of the agent does not.

Another argument is that laboratory data is unrealistic and not useful if it doesn't duplicate potential human exposure. Nothing fills that criterion except accidental human exposure, which we do not need. Experience has given us considerable (albeit not perfect) confidence in our ability to estimate field consequences from laboratory findings. The concept of dose dependent response has been proven valid, although there are admittedly possible discrepancies at the very low dose range. In any case, without the expenditure of enormous amounts of time and money, we cannot have studies that precisely duplicate field exposure.

It is possible to estimate a TCDD exposure rate that will not cause effect in the most sensitive parameters now known to be affected by TCDD. In rats, 0.001 µg/kg/day 5 days a week for 13 weeks, totalling 0.065 µg caused no detectable effect. Guinea pigs given 0.008 µg/kg/weekly for eight weeks also were not affected. Existing data from monkey

experiments has not produced a "no observed effect" dose because the detailed analyses available only through interim sacrifice have not been made in primates. The group of monkeys studied in Allen's laboratories first showed clinical symptoms after three months of exposure to about 0.01 µg TCDD/kg/day, or a total dose of somewhat less than 1 µg/kg, but there is no way of knowing whether pathologic examination would have detected changes at the more than 10-fold lower dose found ineffective in the rat and guinea pig. The existing literature also does not provide access to a useful single dose no observable effect level. There is no useful data that relates specifically to human effects.

It therefore seems reasonable to accept 0.06 µg/kg as a subchronic total dose over a short term, say, one year below which no effect can be detected. From this point it follows that some attempt must be made to judge potential human exposure levels. It is clearly impossible to directly evaluate acquisition by surface contact with treated foliage. Direct analytical methods for TCDD are probably more sensitive than for any other organic compound, but measurement of foliar distribution cannot be made at field application levels.

In my opinion significant TCDD exposure by inhalation is highly improbable. An intake of 0.02 µg of TCDD would require inhalation of one g 2,4,5-T containing 0.02 ppm TCDD, along with 10 g of diluent at usual dilution rates. The volume of air in which the spray is distributed is very large. Such an intake for a 50 kg person would constitute 1/150 of the assumed no observable effect dose. The potential for inhalation of TCDD formed during combustion of 2,4,5-T is infinitely less, because of the enormous dilution in air.

Oral exposure in drinking water is also extremely unlikely because TCDD partitions from water to sediment very efficiently. With dilution in flowing water, TCDD at levels deposited during spraying disappears quickly. Furthermore, TCDD deposited on soil and surfaces binds tightly, and while some material can wash off of leaves, once on soil it remains, neither leaching downward or migrating toward water courses.

An acute spill of large amounts of undiluted spray formulation into a water course could present an isolated hazard but 2,4,5-T and its carriers are objectionable enough in taste and smell that such an accident provides its own alarm. However, a theoretical but remote possibility in such an accident is the selective extraction of TCDD onto sediment, followed by very slow loss of TCDD from sediment to water over a sustained period.

Probably the only way such an accident can occur is through a vehicular accident resulting in loss of herbicide drums into a water course.

Oral intake through consumption of meat or milk presents at least theoretical possibility that an individual might consume enough deer meat containing, say, 10 ppt TCDD to acquire a significant burden of the dioxin. The question then becomes one of the probability that such a circumstance can come to pass. TCDD in concentrations of this order have been found in cattle grazing on 2,4,5-T treated range land; we may assume that an occasional deer can accumulate similar amounts if forage is poor and the animals must eat forage that they have been observed to discriminate against. If uniformly distributed in all edible tissues the amount of TCDD in a pound of meat would be about 4.5 ng. The no observed effect level in a 50 kg human is assumed at 3.0 µg. To reach that amount would require nearly 666 meals of one pound of meat, or the meat from 5-10 deer,

and assumption of perfect retention of TCDD or its effect. In point of fact, the high TCDD concentrations are in liver and fat and such a hypothetical whole animal concentration would not be approached.

A slightly different approach by Dr. George Streisinger makes assumptions about fat concentration and the percentage of fat in, as an example, ground meat. He has concluded that either 78 or 408 (depending on different no-effect assumptions) half-pound meals would exceed the danger barrier. Dr. Streisinger included a 100/1 safety factor, however, and the specified number of meals would bring a person to within 1/100th of a possibly harmful intake.

2,4,5-T is applied on a given forest area only 1-3 times in a timber growth cycle. While it is possible for a deer to acquire significant residues in a single year, the likelihood of a long-term continuous exposure of deer would require migration among sprayed plots, or repeated treatment of a single area, and is therefore remote. Even range land is not usually treated annually.

The significance of TCDD in mothers milk is considerably greater than consumption in meat. The analyses by Dr. Meselson's group indicate the possibility that TCDD on the order of 1-2 ppt may be present in human milk. Dr. Meselson has been careful to state that while he has great confidence in the individual analyses, they are close to detection limits, and the data really asks rather than answers the question.

For the sake of discussion, a sustained output of 1 ppt in human milk could provide an infant dose approaching the no observed effect level. Assuming a liter of milk consumed daily at 100 days, total intake would be 0.1 μg. If the average weight of the infant were 6 kg, the 100 day

dose would be 0.016 µg/kg, or about 1/4 of the no observed effect level. Exposure for 200 days would produce a somewhat lower dose per unit weight because infant weight increases but milk output may not.

It is appropriate to inquire how a human mother can acquire enough TCDD to excrete 1 ppt or 1 ng/L in milk. If a constant input by consumption of one pound of meat daily is assumed, the individual will take in 4.5 ng daily.

Almost all ingested TCDD is excreted in feces, with a minor component excreted in urine. Most chlorinated hydrocarbons emerge in milk to a limited extent, and it is not unexpected that TCDD should be found at low levels in milk. A pound of meat containing 10 ppt TCDD ingested daily would bring into the body 4.5 ng daily. As with any chemical, an equilibrium will eventually be reached where excretion and metabolism of the chemical will equal intake. In the case of TCDD, metabolism to other forms is minimal. There are not data on the fraction of TCDD excretion in milk, but it may be expected to be quite small. If we assume a high figure of 20%, however, an output of about 1 ppt is at least possible. The possibility of such sustained intake of TCDD is remote, however, requiring consumption of 2-3 deer over a 6 month period.

An alternative is a more massive exposure over a short period. We have no knowledge of the distribution into milk of, for example, 1 µg TCDD/kg as a single dose. We do know, however, that TCDD has a half-time of residence in the body of no more than 30 days, and probably closer to 20 days. Milk concentrations will decrease at the same rate as tissue residues, if not faster, and a 200 day nursing period covers more than 6 half-times. A one ppt concentration in milk arising from a single exposure will dwindle to 0.5 ppt in 20-30 days, to 0.25 ppt in 40-60 days, and

0.12 ppt at 60-90 days. Exposure of a nursing infant will therefore be minimal.

It appears to me therefore that the potential for harmful exposure of infants through human milk should be negligible. This route is nonetheless the most probable of the several potential means of contact, and demands a thorough analysis of human milk in exposed populations.

The data on TCDD carcinogenic potential suggest that it is not a carcinogen. It has been suggested that TCDD is a promoter of carcinogenic activity on the basis of a wide variety of tumor types developed over a long exposure period by the Wisconsin group. The recently completed Dow Chemical Co. study indicates that tumor incidence changed only at doses that were lethal to many animals over the two-year test period. The data base exists for one and probably several human epidemiological studies which could answer the question of influence on human cancer incidence. A recommendation is made later for such studies.

Of the toxicological data evaluated, perhaps the most disturbing is that of Allen et al. (1977) suggesting that the lethal dose in monkeys is similar whether the agent is administered over a short period or over several months. Time independence of dosage has never been demonstrated for a chemical. If it truly exists for TCDD, there is implication that the compound leaves permanent damage, even though it has departed from the body. In this situation, continual low level impact would cause gradually accumulating injury until clinical illness would prevail. In that sense, the idea of a no-effect level would not be applicable, and no exposure would be permissible. As with radiation, however, a small intake is possible without endangering health over a lifetime. In the case of TCDD this amount is not known, but it is finite. In view of a

long history of use without apparent injury and an apparently rapidly improving data base, it seems justifiable to allow use of 2,4,5-T and silvex under conditions that will permit virtually no inadvertent or unknowing exposure. It appears to be practical to diminish ambient contact to levels which will have no significance, accepting minor one-time defects in the system which should also have no consequences, and to allow individuals the informed opinion of exposure.

Such a stringent condition is not necessarily incompatible with use of a necessary herbicide. It seems possible to design application methods and associated policies that would prevent involuntary and inadvertent exposures of humans to TCDD.

The present knowledge of dioxin effects and behavior is not sufficient to make an unqualified statement of reasonable safety. Several areas of needed research, suggested in a later segment of this report, should improve our level of confidence. Nonetheless, it is my opinion that 2,4,5-T containing less than 0.02 ppm TCDD can be used safely in reforestation if certain protective practices listed later are instituted. In brief, these involve absolute avoidance of spray intrusion across property lines, identification and protection of dwellings and water supplies reentry discipline and adequate public information programs.

The acute toxicity of 2,4,5-T is quite low and in itself is not a factor in the environmental hazard potential of the herbicide. This segment is concerned only with 2,4,5-T; the implications of the dioxin contaminant have been considered earlier. A wide variety of specific toxic changes have been found in experiments with acute or short-term repeated administration of high doses of 2,4,5-T.

2,4,5-T is teratogenic, but again, only at high doses. The dose response to 2,4,5-T is such a large fraction of the maternal lethal dose is required to produce birth defects.

There are substantial differences in effective teratogenic doses among strains of mice. The lowest effective dose is still substantial, however, and the kind of teratogenic response remains the same. The differences among the genetically very specific mouse strains do raise the possibility of high individual sensitivity in the heterogeneous human population. This question applies to any potential effect in humans by any chemical and really constitutes a common social question that we have not learned to handle.

The excretion of 2,4,5-T by mammals is rapid, with relatively little conversion to other compounds. Initial residues after application are high enough to possibly cause some temporary slight tissue deposition of 2,4,5-T in tissues of grazing animals, if sustained for a period of more than two weeks, but environmental degradation of 2,4,5-T is rapid and the chemical does not migrate extensively, once deposited. For these reasons and because general toxic responses to the herbicide only occur at high doses, I do not consider that 2,4,5-T as used properly in forestry procedures represents a general toxic or teratogenic hazard to the human population.

The bulk of evidence available at this time indicate that 2,4,5-T is not carcinogenic or mutagenic. It must be emphasized that this evidence really assures us only that the herbicide is not highly carcinogenic. An epidemiological study of humans occupationally exposed has also been negative, although other herbicides were found in the same study to be possibly associated with an increased incidence of tumors.

Mutagenic assessment in a number of experimental systems has shown no evidence of such change, and epidemiological study of 2,4,5-T production employees has shown no evidence of cytogenetic change.

I do not believe the issue of carcinogenesis by 2,4,5-T can be arbitrarily closed yet, although at this time it appears that there is not a carcinogenic or mutagenic hazard at exposure levels encountered following typical application. I have made recommendations relating to needed investigations which should establish a more specific level of confidence in that opinion, or possibly cause it to change. An industrial study of carcinogenic effect of a two-year treatment of rats with 2,4,5-T is almost complete and should also shed more light on the issue.

I do not believe that 2,4,5-T per se as used in forestry constitutes a human health hazard.

Hazard Assessment--2,4-D

The acute toxic dose of 2,4-D is quite high, but a number of individuals occupationally exposed to 2,4-D have suffered severe neuromuscular and gastrointestinal effects. These incidents have been the result of gross mishandling of the chemical and are not a part of the environmental health issue.

The reproductive effects of 2,4-D do not appear until doses approaching the lethal level are reached, and there seems to be no reason for concern in this province.

As a potential carcinogen, 2,4-D has had very little study. The only evaluation of which I am aware was negative, in one species, which indicates that the compound is not highly carcinogenic. This finding of course can give no confidence about low level carcinogenicity. Several studies of mutagenic potential have been negative, adding some confidence.

Perhaps the key information lies in the rapid excretion of intact 2,4-D in the urine. This process is rapid and relatively complete. In some species a portion of the compound is converted to other forms for excretion, but the process is rapid. Rapid excretion probably is the factor that prevents 2,4-D or its products from reaching the detectable margin in milk after field exposure. It is possible experimentally to overload an animal to the extent that the material will appear in milk.

Because of its short persistence in the environment and the absence of toxic responses at any doses that might be found in the field, I believe the uses of 2,4-D in practices presently considered standard is not hazardous.

There are, as with any chemical, some open questions, which are addressed in the section on recommendations. These relate primarily to epidemiological needs and clinical surveillance.

Hazard Assessment--Silvex

The general considerations applicable to 2,4,5-T may also be applied to silvex. There are some minor differences between the compounds, but the ranges of toxicity, the manner of biological disposition, and the

environmental behavior are similar enough that similar standards should apply. In the absence of data on silvex, findings from studies of 2,4,5-T should be considered applicable.

As with 2,4,5-T, the issue of TCDD contamination overshadows the characteristics of silvex itself. The one mitigating circumstances is the more limited distribution of silvex and therefore less extensive distribution of TCDD.

It is my opinion that present uses of silvex do not constitute a hazard, from silvex itself. The issue of the TCDD contaminant is separately addressed. There is, however, further information needed to increase confidence in that opinion, and recommenations have been made in the appropriate section.

RECOMMENDATIONS OF STEPS WHICH SHOULD BE CONSIDERED TO ASSURE THAT EXPOSURE TO TCDD IS MINIMIZED OR PREVENTED

I have stated my opinion that 2,4,5-T can be used safely, with certain additional safeguards. I believe that opinion should be reviewed as new data emerges. A number of clinical questions now being asked have accessible answers, and these must be obtained. In addition, the toxic nature of TCDD and our inability to measure it satisfactorily in the environment dictates a very conservative attitude about its distribution. I see no inconsistency in accepting its presence in small quantities and recommending unique steps to prevent those quantities from reaching people.

To assure public protection and a more compatible relation with the public, I suggest the following considerations in designing a spray program.

1. With the consideration of health hazard, there is a political reality that needs more attention. Every treatment operation must be designed to prevent intrusion of spray onto premises not under control of the agency or firm using the chemical, as a matter of principle. I would not be surprised if Forest Service policy includes that concept, but I would also be surprised if its application is somewhat less than perfect. Personal rights are becoming more and more clearly defined, and it seems to be time to decide where such rights begin and end, and take a visible public position on the issue.

2. As a means of preventing any involuntary exposure, there should be assurances that every residence is identified, with any water sources that may be in the Forest. Non-legal residents should also be identified.

Questions of liability and informed consent and other relationships with the Forest Service ought to be clearly laid out.

3. Present buffer procedures should be examined in detail and publicly shown to be adequate. If a 100-foot zone cannot be proven certainly safe, it should be expanded.

4. An intensive posting and advertising program should be used to notify the public where herbicide treatments are located. The information should be directed toward special users of the area, such as hunters, as well as the adjacent population.

5. There is definite need for documented assurance that applied herbicides arrive only at the intended target area. If this requires a spot monitoring program during and after application it should be instituted. Here again is an issue of public confidence; precision of application may be very high, but no one outside the industry knows about it, if it is so.

RESEARCH NEEDS TO MORE CERTAINLY ESTABLISH THE SAFETY OF PRESENT USES OF PHENOXY HERBICIDES

1. During discussions of herbicide use issues we are confronted by innumerable allusions to people made ill by application of the agents. These incidents range from word of mouth descriptions of a neighbor's experience to an extensive series of carefully constructed interviews with Vietnamese who were subjected to herbicides as chemical warfare. As they appear in the context of our forestry practices, these anecdotal descriptions of illness cannot be documented and are of little value in assessing toxic hazard. They are of concern, nonetheless.

A clinical surveillance team should be commissioned to evaluate immediately any alleged herbicide contact incidents. There is no doubt

in my mind that people complaining of physical injury are in fact affected. I am skeptical that the application of herbicide causes these effects in more than a few incidents, but it is possible. Of more importance, there may be some other public health hazard operant in the area which causes the symptoms. It is even possible that other illness is brought to the attention of the victims by their immediate concern about improper herbicide intrusion on their private property.

2. The opinion of Van Miller et al. (1977) that TCDD is a promoter of carcinogenic effect by other compounds seems reasonable according to their evidence. If the forest application of 2,4,5-T containing TCDD is inserting effective levels of TCDD into the human environment, epidemiological evaluation should disclose higher than normal incidence of various forms of cancer in 2,4,5-T use areas. Lane County, Oregon, has a very effective tumor registry program, with virtually all pathology of human cancer examined by a single consortium of specialists. These data should provide evidence of any existing difference in cancer patterns from other areas. A more definitive study should be possible in rangeland or rice-growing areas where the herbicide is used annually. Epidemiological studies such as these may settle the persistent question of cancer latency, because on an area basis, the pattern of past use of herbicides should be reasonably accessible.

3. A thorough health surveillance should be made of all herbicide applicators and others with industrial or agricultural exposure to 2,4,5-T or silvex. Cytogenetic evaluation through several seasons of the year should be an integral part of the study. Design of the medical components of the survey should be by a nationally constituted panel.

4. Environmental degradation of TCDD is still inadequately understood. Photodegradation by light reflected from or transmitted through foliage must be measured, as must reaction in shade and under cloud cover.

5. With present sensitivities of analysis, translocation of TCDD in plants should be restudied.

6. Affinity of TCDD to plant surfaces should be examined directly, to determine how efficiently the chemical can be removed by surface contact and by animal digestion of forage.

7. Augmentation of industrial efforts to eliminate TCDD from trichlorophenol derivatives seems to be a legitimate governmental concern, and should be encouraged. Elimination of the contaminant would obviously eliminate the issue.

8. A search should be made for chemical additives that would react with TCDD or accelerate its breakdown.

9. The human milk and fat biopsy program being developed by EPA should be encouraged.

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